Of mice and fishes: selection and the maintenance of variation

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Abstract: Understanding how trait variation is maintained within populations is important for predicting how populations will respond to environmental change. This thesis uses observational, experimental, modelling, population and quantitative genetic approaches to investigate proximate and ultimate mechanisms underlying the maintenance of variation in two species of fishes and mice. One important feature unites these four species: they can be observed and captured in the wild, and they can be kept in the laboratory and subjected to controlled experiments. The three conditions under which natural selection can maintain within-population variation were investigated: 1) variants have equal fitness, 2) selection eliminates variation but new variants are continuously generated, and 3) a balance of selective forces. Most of the work here described investigates the latter. Evidence was found for temporal fluctuation in selection in striped mice Rhabdomys pomilio (Chapter 3) and in guppies Poecilia reticulata (Chapter 8), and spatial fluctuation in selection in guppies (Chapter 7) from introduced populations (Chapter 5). We considered negative frequency-dependent selection as a mechanism to maintain rare male colour morphs in Poecilia parae (Chapter 1) and Major Histocompatibility Complex allelic variation in house mice Mus musculus domesticus (Chapter 10). Chapters 10 and 11 investigate selection at different levels at the t haplotype, which is a driving selfish genetic element in house mice. While the t haplotype has an advantage relative to the wildtype allele in transmission to the next generation, thereby experiencing positive selection, it is selected against at the level of the animal because homozygotes die prenatally. However, female heterozygotes do have a survival benefit through heterosis (Chapter 11) and Chapter 12 tests whether this survival difference between genotypes has correlated behavioural effects. Genetic tools to facilitate estimates of selection were developed in Chapter 14. Whether selection influences trait variation in the next generation depends on their proximate bases. Proximate genetic bases of trait variation were investigated in Chapters 1,2, 6 and 15 while proximate environmental bases of trait variation were considered in Chapters 3, 4, 8, 9 and 13. The combination of understanding patterns of selection and the additive genetic versus environmental contributions to trait variation are important in predicting how populations will respond to the environmental challenges of tomorrow.

Of mice and fishes: selection and the maintenance of variation

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Contents

Introduction

About nine million species inhabit the Earth (MORA *et al.* 2011). Within species individuals typically vary in their characteristics, thus the living world is composed of an uncountable diversity of forms. Understanding how this came to be and will change in the future are the main challenges of the field of evolutionary biology. The aim of this thesis is to investigate how contemporary variation is maintained, using two mammal and two fish species, and different kinds of traits.

Variation occurs at the genetic and phenotypic level. What is often measured is phenotypic variation, which is variation in observable characters that result from the interaction between genes and the environment in which they are expressed. For example, males of some species of live-bearing poeciliid fishes show impressive phenotypic variation in colour pattern, and also in body size (Figure 1). These are not local adaptations, with a single well-adapted colour pattern per population, as all occur together within populations in the same habitat. How can this be explained? There are two types of answers, one based on proximate causes (mechanisms) and one on ultimate causes (current adaptation and evolutionary history).

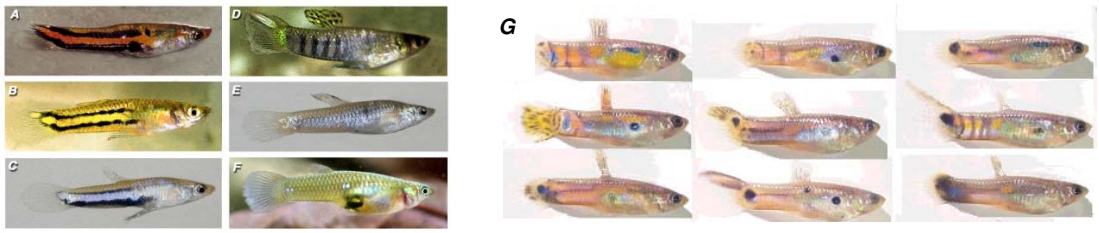

Figure 1. *A-E*. Phenotypic variation in male *Poecilia parae* within a natural population. *F*. Female *P. parae*. Source: Chapter 1. G. Phenotypic variation in male guppies *P. reticulata* from a single feral population in Australia.

In poeciliid fishes, proximate causes of colour pattern variation are differential distribution of pigment cells with different absorbance spectra in the skin (BLANCHARD *et al.* 1991). Pigmentation variation in this family of fishes is mainly the result of genetic differences between individuals at autosomes, X and Y chromosomes (Chapter 2) but is also modulated by environmental differences, such as carotenoid content of the diet (GRETHER *et al.* 1999).

Male-specific colour patterns are under hormonal control and develop near sexual maturity (HOUDE 1997). Ultimate causes of colour pattern variation relate to current and historical patterns of natural selection. Natural selection is the process in which alleles change in frequency between generations depending on the relative survival and reproductive success of organisms bearing those alleles. In the case of *Poecilia parae*, the less spectacularly coloured morphs (to our eyes) make up a high proportion of adult males within populations (Chapter 1), and the high frequency indicates that these morphs must have a selective advantage. Subsequent research has suggested that the nature of this advantage is survival - the less colourful morphs are targeted less by predators than the most brightly coloured morphs (HURTADO-GONZALES *et al.* 2010). This brings up the question of how the more colourful morphs manage to be represented in each generation. Patterns of inheritance in laboratory crossings have indicated that male colour pattern is inherited from father to son on the Y chromosome (Chapter 1). As females do not have the Y chromosome, it takes only one generation in which males of one colour morph fail to reproduce (perhaps by chance) for that morph and the alleles determining it to go locally extinct. This leads to the more general question of how variation is maintained within populations, which is a general theme of my research.

John Maynard Smith (1998) outlined three conditions in which natural selection can maintain variation within populations.

1) Variation is selectively neutral, so that variants have the same fitness. As predators differentially target different colour morphs in *P. parae*, this is unlikely to apply. However, we found an example in striped mice *Rhabdomys pumilio* of equal fitness. Within a single population of striped mice, males pursue one of three reproductive tactics, each associated with behavioural and physiological differences (SCHRADIN *et al.* 2009). How females are distributed in the environment appears to influence which reproductive tactics are adopted in a given year (Chapter 3), and therefore these reproductive tactics are considered to be phenotypically plastic (Chapter 4). Phenotypic plasticity refers to the case where a given genotype produces different phenotypes in different environments. Comparison of tactics between father and son (Chapter 3) do not refute this conclusion as it shows that tactics are clearly not inherited like colour morphs of the fish *P. parae*. We found that variation in tactic

was selectively neutral in one breeding season studied, as different reproductive tactics had equal reproductive fitness (Chapter 3).

2) A balance between selection eliminating variation and mutation adding variation. In this scenario, mutation repeatedly creates less fit variants that are then eliminated by natural selection. Similarly, migration can bring in less fit variants that are selected against. In the case of introduced species, humans have facilitated this process, by deliberately or unwittingly moving organisms between populations or bringing them outside of their natural ranges into new areas. We investigated migration and genetic diversity in introduced populations of guppies in Australia. Guppies are native to South America and the Caribbean (Houde 1997), but have been exported around the world for mosquito control and as pets (Chapter 5). By genetic analyses of guppies sampled from seven populations in Northern Queensland, we showed that contemporary migration within streams occurs, and we could identify likely sources of the introductions (Chapter 5).

3) A balance of selective forces maintains variation, through i) temporal and spatial fluctation in selection, ii) negative frequency-dependent selection, iii) selection acting at different levels, and iv) heterosis.

i) Temporal and spatial fluctuation in selection

Under natural conditions, selection often favours different trait values at different times and in different locations. A classic example is selection on bill size in the Darwin's finch *Geospiza fortis*. Using 30 years of data from the island of Daphne Major, Grant & Grant (2002) found evidence of positive selection in 6 years, and negative selection in 3 years. Bill size has high heritability (KELLER *et al.* 2001), so that the bill variation is due to a large extent to additive genetic variation, the genetic variation that is the result of differences in alleles inherited from parents. With different alleles favoured in different years, bill size should fluctuate over the 30 years of the study, which is what happened (GRANT and GRANT 2002). Thus temporal fluctuation in selection has maintained variation in bill size in this species. We measured selection on striped mouse male reproductive tactics in three years at one study site (Chapter 3). In one season territorial males had a reproductive advantage over males adopting other tactics, in another season two tactics were equally successful, and in a third season all males adopted the same tactic. Comparing within males adopting a particular

tactic, heavier males were not more successful than lighter males, except in the third season. Body size variation in striped mice is expected to be highly to moderately heritable, as it is in house mice *Mus musculus* (FALCONER and MACKAY 1989; LEAMY 1988), and many other species (MOUSSEAU and ROFF 1987) including *P. parae*, in which it is associated with loci on the Y chromosome (Chapter 6). Thus, if variation in the traits of reproductive tactic adopted, or of body weight, have a genetic basis in striped mice, we would expect different alleles or combinations of alleles to be favoured in different years. This would maintain allelic variation within populations.

Spatial variation in selection at a larger scale was investigated in guppies, using introduced populations in Australia. Guppies have elaborate colour patterns (Figure 1), which differ between natural populations in Trinidad partly in relation to predator regime. Where predation is high, males are less colourful on average, because predators target showy males, and females are also less choosy (HOUDE 1997). Guppies were introduced into Australia just over a century ago, and two source populations could be identified (Chapter 5). We investigated divergence in male sexual traits (size, colour spots and sperm numbers) in six introduced Australian populations, in relation to sexual selection, predation and genetic drift (Chapter 7). Male sexual traits differed significantly between populations. Sexual selection, which we measured as differences between males in the number of offspring sired in a competitive arena in relation to male trait values, also differed between populations, as did predation. Thus selection varies spatially, favouring different variants in different populations.

Within populations of guppies, selection may also vary temporally and spatially depending on ambient light spectra. At different times of day, or in shade vs full sun, guppy colour patterns are more or less conspicuous (ENDLER 1991). By using shade cloths and filters, we experimentally altered light spectra in aquarium tanks to mimic conditions in early morning/late afternoon, mid-day in forest shade, and mid-day in woodland shade for guppies in the wild (Chapter 8). Male and female sexual behaviour in the experimental tanks were observed. While females preferred males with more orange in all three treatments, larger body size was preferred in one treatment and smaller body size in others. This suggests that the direction of selection can vary across a day, which would contribute to maintaining variation in male body size.

ii) Negative frequency-dependent selection

If alleles or traits confer an advantage when they are rare, they will increase in frequency and be relatively stably maintained within populations. An example is the bill crossing in two races of red crossbills *Loxia curvirostra* (Figure 2), in which the lower mandible crosses to the right or to the left with equal frequency (BENKMAN 1996). The bill crossing allows crossbills to feed on closed or partly open cones (BENKMAN and LINDHOLM 1991), by inserting the tip of the upper mandible between the scale and the cone and moving the lower mandible sideways. This moves the scale away from the cone, allowing the crossbill to insert its tongue and remove the seed. If it has a crossing to the right, it forages on the left side of the cone, which means that it cannot access seeds on the right side of the cone. This gives an advantage to a left-crossed bird relative to a right-crossed bird foraging later on the same cone, who can as efficiently extract seeds from the same cone as the first bird, while a second right-crossed bird is much less efficient. This provides a negative frequency-dependent advantage to the rarer crossing type (BENKMAN 1996).

Figure 2. The bill crossing of the red crossbill, from Benkman & Lindholm (1991).

A negative frequency-dependent advantage appears to be important in the ability of rare male *Poecilia parae* colour morphs (Figure 1) to persist despite genetic drift and predation (HURTADO-GONZALES *et al.* 2010). The red and yellow colour morphs occur at low frequency within populations (Chapter 2). As females have a preference for males of the red and yellow morphs (Chapter 2), these rare morphs are likely to gain a reproductive advantage through sexual selection.

A further system in which we expect negative frequency-dependent selection to occur is in major histocompatibility complex (MHC) variation. MHC genes code for cell receptors that bind to foreign antigens and present them to T cells, thereby initiating an immune cascade against pathogens (KLEIN 1986). The MHC alleles that will be positively selected include those that increase resistance to the pathogens and parasites that currently afflict a population (BERNATCHEZ and LANDRY 2003). Sexual selection should reinforce this pattern, as resistant

individuals are more likely to be chosen as mating partners (HAMILTON and ZUK 1982; KAVALIERS and COLWELL 1995). However, when conditions change, rare alleles that improve resistance to the new disease-causing organisms will be selected for, leading to cycles of positive and negative selection (CLARKE and KIRBY 1966; HAMILTON and ZUK 1982). In a long-term study of a wild house mouse *Mus musculus domesticus* population from Illnau, Switzerland (Chapter 9), we characterised MHC variation at two loci over time. In 29 mice sampled from 2002 – 2004, 5 alleles were found at one locus and 4 at another (Chapter 10). In 2012, in 60 mice, only 2 alleles per locus were found (Manning, unpublished MSc thesis). In this case, negative frequency-dependence has apparently not maintained MHC variation.

iii) Selection acting at different levels

In theory, natural selection can act at a number of levels of biological organisation, from alleles, to organisms, societies and species (OKASHA 2006) and different variants can be effective in competition with others at different levels. We found selection to be occurring at the gene level as well as at the organismal level in a set of genes in the house mouse called the *t* haplotype. The idea that natural selection occurs at the level of the gene can be illustrated by cases in which Mendelian segregation is disrupted. Mendelian segregation ensures that there is a 50% chance that any given allele in a diploid organism is transmitted vertically from parent to offspring. An allele that can consistently increase its representation in zygotes above 50% (a process called drive) will have an advantage over other alleles at the same locus. The "unfairness" of this bias in inheritance is reflected in the terminology used to identify such alleles (or haplotypes if they are a set of linked alleles that show very little recombination): selfish genetic elements (SGEs). The *t* haplotype, which is a large SGE comprised of four large inversions taking up a total of a third of chromosome 17 of house mice (LYON 2003), is a relatively well-studied example. Numerous variants of the *t* haplotype have been discovered in the last 80 years (LYON 2003), but our work focusses on a *t* haplotype variant found in a free-living study population of wild house mice from Illnau, Switzerland (Chapter 9). This *t* haplotype shows 90% drive in males, so that 90% of offspring of heterozygous males inherit the *t.* In females, the *t* haplotype shows normal Mendelian inheritance (Chapter 10). The sex difference occurs because the *t* is active in the testes during sperm development. Drive-causing genes (called distorters) within the *t* haplotype cause signalling cascades that impair sperm motility, while another linked gene (called the

responder) rescues the impairment of *t* bearing sperm (HERRMANN and BAUER 2012) in a poison-antidote system. Thus within the same (*t* heterozygous) male, wildtype sperm have impaired motility and are handicapped in their ability to fertilise eggs, causing *t* bearing sperm to fertilise the lion's share of eggs in monogamous matings. The *t* haplotype is prevented from fixing within populations because it carries a recessive lethal allele that causes prenatal death (Chapter 10). Homozygotes are therefore eliminated in every generation. Combining the properties of 90% drive (positive selection at the gene level) and homozygote lethality (negative selection at the zygote level) leads to theoretical expectations that the frequency of the *t* haplotype will remain high (Figure 3). However, there is a mismatch between expectations and the observed data, as the *t* frequency declined in frequency over time in our study population. A possible explanation is inbreeding, as it increases the frequency of homozygotes, and therefore would reduce *t* frequency (DUNN 1957) but we could eliminate this as a cause of the *t* frequency decline (Chapter 11), leaving net selection against the *t* haplotype as the most likely cause of the decline. We aim to understand in detail how natural selection acts on the *t* haplotype both at the level of the individual animal and the level of the gene. In this case, selection is not

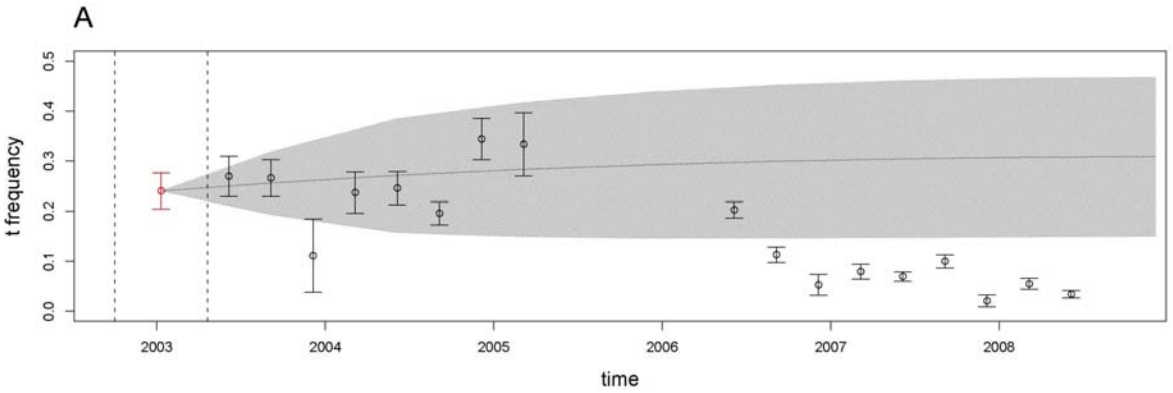

Figure 3. Observed and expected frequency of the *t* haplotype (with standard error) of wild house mice at the Illnau study population. In red, in 2003, is the *t* frequency of the first 50 adults in the population. In black are the *t* frequencies of pups born into the population, grouped in three month intervals. The grey line shows predicted *t* frequencies and their 95% confidence envelopes based on a model incorporating drive and homozygote lethality. From Chapter 11.

maintaining genetic variation in the population, but removing it. The two most promising avenues are precopulatory mate choice (LENINGTON and COOPERSMITH 1992; Chapter 10) and postcopulatory sexual selection, through sperm competition between males (Chapter 11), and possibly by sperm selection by females (Chapter 10).

iv) Heterosis

Heterosis is the advantage that heterozygous individuals have over homozygotes (overdominance). The best-known example of a stable polymorphism due to heterosis is sickle-cell hemoglobin, a hemoglobin variant in humans that protects against malaria but is susceptible to anemia. Heterozygotes of this variant are protected against malaria and anemia, but homozygotes have serious anemia (STEARNS and HOEKSTRA 2000). In house mice, we documented heterosis in that *t* heterozygous females have a survival advantage over wildtype females (Chapter 11). Modelling this effect shows that heterosis improves the ability of the *t* haplotype to spread within a population (Chapter 11). However, this advantage did not result in the maintenance of the *t* haplotype within the study population of house mice (Figure 3).

The association between the *t* haplotype and increased longevity in females may be due to a phenotypic or genetic correlation between activity levels and longevity. A well-documented phenotypic correlation is that between number of offspring and body size, with increasing body size increasing the number of offspring. Additional growth is required to increase body size, but this delays time to sexual maturity, and thus decreases the chances of surviving until maturity (ROFF 2007). Thus offspring number and body size trade-off against mortality. In a similar vein, there is a negative relationship between daily energy expenditure and longevity (SPEAKMAN *et al.* 2002). As *t* heterozygous females have higher longevity than the wildtype (Chapter 11), we predicted that *t* heterozygous females would have behavioural traits associated with low energy expenditure (lower activity, lower metabolic rate, reduced exploration, reduced boldness; (CAREAU *et al.* 2008)) compared to wildtypes. Using laboratory behavioural tests, we found partial support for our predictions in that *t* heterozygous females were less active and tended to consume less food (Chapter 12). This correlation possibly has a genetic basis, with a locus or loci in linkage disequilibrium with the *t* conceivably influencing survival and/or activity of females. Whatever the cause, this correlation could maintain variation in activity levels within populations when individuals

differ in their survival expectations, if fitness does not differ between these variants (although Figure 3 suggests that it does), or if selection on these variants varies sufficiently in direction in space or time. As cause and effect are unclear, the converse may also apply: variation in survival may be maintained when individuals differ in their activity levels. Trade-offs could be involved in this system; low activity and high survival could trade-off against slower growth rate and greater age at sexual maturity. This has yet to be fully investigated.

Approach

Finding an appropriate question requires an understanding of the natural history of the organism to be studied, and preferably field observations. For example, we study free-living wild house mice in a long-term field project. As we saw that at any one time, numerous females were apparently not pregnant or lactating, we started an investigation into female reproductive competition (Chapter 9). Alternatively, questions can carry over from one system to another. In studying responses of hosts to brood parasitism by cuckoos in my doctoral research, I became interested in questions of how variation is maintained (or not) within natural populations. When cuckoos eggs are a good match to host eggs, then it is very difficult for hosts to recognise parasitic eggs. Negative frequency-dependent selection can favour divergence of host eggs within a population, as cuckoo eggs are unlikely to match rare host egg colour types. An interest in the mechanisms allowing variation in egg colour to be maintained within populations led me to the study of variation in male colour patterns within natural populations of the fish *Poecilia parae*. This links directly to investigations of the factors influencing variation in social behaviour and the frequency of a selfish genetic element within a wild mouse population.

Methods

Much of my resarch has relied on fieldwork and laboratory experiments, particularly to measure selection. I have usually measured natural selection by the covariance between the relative number of offspring produced and the trait of interest, as in Chapters 3, 7, 10 and 13. Determining the relative number of offspring produced or number of mates is not trivial in settings in which males and females are free to mate with numerous partners. It requires extensive genetic sampling of offspring and of potential parents together with parentage analysis using variable genetic markers to match offspring to their parents. For each species,

appropriate genetic markers must be found or developed. We developed new polymorphic microsatellite markers for use in analysis of reproductive success of males in *P. parae* (Chapter 14) and identified sets of markers for use in house mice (Chapter 10 and 11) and striped mice (Chapters 3 and 13).

Behavioural vs morphological traits

Studies of animal behaviour are more challenging than studies of easily quantifiable and reliable discrete morphological traits, such as *P. parae* colour patterns, because behaviour is highly influenced by environmental conditions. In a review of heritabilities, Mousseau and Roff (1987) found that life history traits have the lowest average heritabilities, followed by behavioural and physiological traits, while morphological traits have the highest heritabilities. In guppies, a three-generation artificial selection experiment on female preferences and male attractivenes showed evolutionary responses in some aspects of male colour patterns, but none in female preferences (Chapter 15). This is consistent with higher heritabilities of morphological traits compared with behavioural traits. Low heritabilities imply high influence of the environment on phenotypes. In striped mice, the reproductive tactic expressed by a male is thought to be determined by environmental conditions, rather than by genetic variation (Chapter 4). If this is strictly the case, then differences in reproductive success due to tactic adopted will not result in evolutionary change. It seems more likely however that the ability of males to respond appropriately to environmental conditions is variable, and caused by genetic variation, as is the case in timing of reproduction in great tits (NUSSEY *et al.* 2005).

Environmental influences on behaviour can be profound. Aggressive behaviour in striped mice is influenced by season, reproductive tactic and whether the opponent is a neighbour or not (Chapter 13). Aggression is a special example, as are courtship, communication and cooperative behaviours, as they are social behaviours. In social behaviours other individuals comprise the environment. As expression of behaviour in one individual is influenced by that of another, understanding variation in social behaviour is especially challenging (WOLF *et al.* 1999). Such indirect effects are often ignored, but can be explicitly modelled (MOORE *et al.* 1997).

Environmental influences on phenotypes can be cross-generational and act at different developmental stages. The maternal environment can indirectly influence the phenotype of offspring, independent of offspring genotype, through maternal effects. For example, offspring body size in the livebearing fish *P. parae* is heavily influenced by maternal effects at birth, which diminish over time (Chapter 6). Maternal effects are also expected on social behaviour (CHEVERUD and MOORE 1994).

In summary, my research combines observations and experiments in the field and in the laboratory to understand current selection on behavioural and morphological traits, with some investigation into the proximate cause of traits. These are together needed to understand not only how variation is currently maintained, but also to provide insights into historical and expected future changes in traits that are present today, or in changes of reproductive success should traits stay the same but the pattern of selection differs. Understanding how selection acts on traits, combined with information on their genetic basis, allows us to predict how traits will change in the near future, under controlled conditions (for the subsequent 10-20 generations (ROFF 2007)). Models of change based on selection and estimates of heritability are however often wrong in natural populations, because they fail to account properly for environmental effects (e.g. GRANT and GRANT 2002; OZGUL *et al.* 2009). Thus a better understanding particularly of temporal and spatial fluctuation in selection in response to environmental change is of particular importance in an age of intense human modification of the environment.

Summary

Understanding how trait variation is maintained within populations is important for predicting how populations will respond to environmental change. This thesis uses observational, experimental, modelling, population and quantitative genetic approaches to investigate proximate and ultimate mechanisms underlying the maintenance of variation in two species of fishes and mice. One important feature unites these four species: they can be observed and captured in the wild, and they can be kept in the laboratory and subjected to controlled experiments. The three conditions under which natural selection can maintain within-population variation were investigated: 1) variants have equal fitness, 2) selection eliminates variation but new variants are continuously generated, and 3) a balance of selective forces. Most of the work here described investigates the latter. Evidence was found for temporal fluctuation in selection in striped mice *Rhabdomys pomilio* (Chapter 3) and in guppies *Poecilia reticulata* (Chapter 8), and spatial fluctuation in selection in guppies (Chapter 7) from introduced populations (Chapter 5). We considered negative frequency-dependent selection as a mechanism to maintain rare male colour morphs in *Poecilia parae* (Chapter 1) and major histocompatibility complex allelic variation in house mice *Mus musculus domesticus* (Chapter 10). Chapters 10 and 11 investigate selection at different levels on the *t* haplotype, which is a driving selfish genetic element in house mice. While the *t* haplotype has an advantage relative to the wildtype allele in transmission to the next generation, thereby experiencing positive selection, it is selected against at the level of the animal because homozygotes die prenatally. However, female heterozygotes do have a survival benefit through heterosis (Chapter 11) and Chapter 12 tests whether this survival difference between genotypes has correlated behavioural effects. Genetic tools to facilitate estimates of selection were developed in Chapter 14.

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References

BENKMAN, C. W., 1996 Are the ratios of bill crossing morphs in crossbills the result of frequency-dependent selection? Evolutionary Ecology **10:** 119-126.

BENKMAN, C. W., and A. K. LINDHOLM, 1991 The advantages and evolution of a morphological novelty. Nature **349:** 519 -520.

BERNATCHEZ, L., and C. LANDRY, 2003 MHC studies in nonmodel vertebrates: what have we learned about natural selection in 15 years? Journal of Evolutionary Biology **16:** 363-377.

BLANCHARD, P. D., R. A. ANGUS, R. L. MORRISON, S. K. FROST-MASON and J. H. SHEETZ, 1991 Pigments and ultrastructures of pigment cells in xanthic sailfin mollies (*Poecilia latipinna*). Pigment Cell Research **4:** 240-246.

CAREAU, V., D. THOMAS, M. M. HUMPHRIES and D. RÉALE, 2008 Energy metabolism and animal personality. Oikos **117:** 641-653.

CHEVERUD, J. M., and A. J. MOORE, 1994 Quantitative genetics and the role of the environment provided by relatives in behavioral evolution, pp. 67 - 100 in *Quantitative genetic studies of behavioral evolution*, edited by C. R. B. BOAKE. University of Chicago Press, Chicago.

CLARKE, B., and D. R. S. KIRBY, 1966 Maintenance of histocompatibility polymorphisms. Nature **211:** 999-1000.

DUNN, L. C., 1957 Evidence of evolutionary forces leading to the spread of lethal genes in wild populations house mice. Proceedings of the National Academy of Sciences **43:** 158-163.

ENDLER, J. A., 1991 Variation in the appearance of guppy color patterns to guppies and their predators under different visual conditions. Vision Research **31:** 587-608.

FALCONER, D. S., and T. MACKAY, 1989 *Introduction to Quantitative Genetics*. Pearson Education, Harlow, Essex.

GRANT, P. R., and B. R. GRANT, 2002 Unpredictable evolution in a 30-year study of Darwin's finches. Science **296:** 707-711.

GRETHER, G. F., J. HUDON and D. F. MILLIE, 1999 Carotenoid limitation of sexual coloration along an environmental gradient in guppies. Proceedings of the Royal Society of London B **266:** 1317-1322.

HAMILTON, W. D., and M. ZUK, 1982 Heritable true fitness and bright birds: a role for parasites? Science **218:** 384-386.

HERRMANN, B. G., and H. BAUER, 2012 The mouse *t*-haplotype: a selfish chromosome - genetics, molecular mechanism, and evolution, pp. 297-314 in *Evolution of the House Mouse*, edited by M. MACHOLÁN, S. J. E. BAIRD, P. MUNCLINGER and J. PIÁLEK. Cambridge University Press, Cambridge.

HOUDE, A. E., 1997 *Sex, Color, and Mate Choice in Guppies*. Princeton University Press, Princeton.

HURTADO-GONZALES, J. L., D. T. BALDASSARRE and J. A. C. UY, 2010 Interaction between female mating preferences and predation may explain the maintenance of rare males in the pentamorphic fish *Poecilia parae*. Journal of Evolutionary Biology **23:** 1293-1301.

KAVALIERS, M., and D. D. COLWELL, 1995 Discrimination by female mice between the odours of parasitized and non-parasitized males. Proceedings of the Royal Society of London. Series B: Biological Sciences **261:** 31-35.

KELLER, L. F., P. R. GRANT, B. R. GRANT and K. PETREN, 2001 Heritability of morphological traits in Darwin's Finches: misidentified paternity and maternal effects. Heredity **87:** 325-336.

KLEIN, J., 1986 *Natural History of the Major Histocompatibility Complex*. John Wiley and Sons, New York.

LEAMY, L., 1988 Genetic and maternal influences on brain and body size in randombred house mice. Evolution **42:** 42-53.

LENINGTON, S., and C. COOPERSMITH, 1992 Genetic basis of mating preferences in wild house mice. American Zoologist **32:** 40-47.

LYON, M. F., 2003 Transmission ratio distortion in mice. Annual Review of Genetics **37:** 393-408.

MAYNARD SMITH, J., 1998 *Evolutionary Genetics*. Oxford University Press, Oxford.

MOORE, A. J., E. D. BRODIE and J. B. WOLF, 1997 Interacting phenotypes and the evolutionary process. 1. Direct and indirect genetic effects of social interactions. Evolution **51:** 1352-1362.

MORA, C., D. P. TITTENSOR, S. ADL, A. G. B. SIMPSON and B. WORM, 2011 How many species are there on Earth and in the ocean? PLoS Biology **9:** e1001127.

MOUSSEAU, T. A., and D. A. ROFF, 1987 Natural selection and the heritability of fitness components. Heredity **59:** 181-197.

NUSSEY, D. H., E. POSTMA, P. GIENAPP and M. E. VISSER, 2005 Selection on heritable phenotypic plasticity in a wild bird population. Science **310:** 304-306.

OKASHA, S., 2006 *Evolution and the Levels of Selection*. Clarendon Press, Oxford.

OZGUL, A., S. TULJAPURKAR, T. G. BENTON, J. M. PEMBERTON, T. H. CLUTTON-BROCK *et al.*, 2009 The dynamics of phenotypic change and the shrinking sheep of St. Kilda. Science **325:** 464-467.

ROFF, D. A., 2007 Contributions of genomics to life-history theory. Nature Reviews Genetics **8:** 116-125.

SCHRADIN, C., M. SCANTLEBURY, N. PILLAY and B. KÖNIG, 2009 Testosterone levels in dominant sociable males are lower than in solitary roamers: physiological differences between three male reproductive tactics in a sociably flexible mammal. American Naturalist **173:** 376-388.

SPEAKMAN, J. R., C. SELMAN, J. S. MCLAREN and E. J. HARPER, 2002 Living fast, dying when? The link between aging and energetics. The Journal of Nutrition **132:** 1583S-1597S.

STEARNS, S. C., and R. F. HOEKSTRA, 2000 *Evolution: an introduction*. Oxford University Press, Oxford.

WOLF, J. B., E. D. I. BRODIE and A. J. MOORE, 1999 Interacting phenotypes and the evolutionary process. II. Selection resulting from social interactions. American Naturalist **153:** 254-266.

Chapter 1

Extreme polymorphism in a Y-linked sexually selected trait

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Extreme polymorphism in a Y-linked sexually selected trait

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Males of the livebearing fish, *Poecilia parae*, exhibit one of the most complex polymorphisms known to occur within populations, whereas females are monomorphic. We describe five distinct male colour morphs and an associated size dimorphism, and demonstrate through pedigree analysis that the locus or loci controlling the male colour polymorphism is linked to the Y-chromosome. Field surveys from 1999 to 2002 of nine populations in Guyana and Suriname, South America, indicate that some morphs are consistently abundant and others are rare, implying that the colour polymorphism has important fitness consequences. By rearing offspring of field-inseminated females, we showed that the common morph is also the most successful morph in terms of reproduction. However, dichotomous choice tests show that two rare morphs are preferred by females over the common morph. These results suggest that alternative male mating strategies, sperm competition, overt male–male competition, or other processes are overriding female preferences in these populations. Furthermore, Y-linkage of the colour polymorphism in *P. parae* supports the hypothesis that heterogametic sex chromosomes harbour sexually antagonistic traits beneficial to the heterogametic sex.
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Keywords: genetic polymorphism; Y-chromosome; *Poecilia parae*; sexual selection

Introduction

Understanding the maintenance of variation that occurs in discrete morphs is a classic question in evolutionary genetics (eg Fisher, 1931; Clarke, 1962). Several basic questions must be answered in order to understand the maintenance of such polymorphisms. To what extent are they environmentally or genetically controlled? If genetically controlled, what are the detailed genetic mechanisms underlying the variation (eg, number of loci, linkage patterns)? What are the temporal and spatial distributions of morphs? And what are the fitness consequences of the different types?

Models of sexual selection (eg Lande, 1981; Rice, 1984) and reproductive isolation (eg Kondrashov and Kondrashov, 1999) have shown the importance of understanding the genetic basis of characters involved in these processes, and therefore there has been much recent interest in the genetic architecture of sexually selected traits (eg Reinhold, 1998: Ritchie and Phillips, 1998; Lande and Wilkinson, 1999; Sinervo and Svensson, 2002). For example, linkage patterns may facilitate or constrain genetic correlations underlying sexual selection processes (Lande and Wilkinson, 1999; Lindholm and Breden, 2002). Furthermore, fitness consequences such as those due to sexual selection can influence the linkage patterns themselves. Sexually antagonistic traits, those that are beneficial to one sex but detrimental to the other, are often inherited on sex chromosomes rather than autosomes (Reinhold, 1998). Sexually antagonistic traits that benefit the heterogametic sex are thought to evolve more easily when linked to the heterogametic sex chromosome (Y or W), as they are not subject to selection in the other sex (Fisher, 1931; Rice, 1987). Thus, determining the genetic basis and linkage patterns of sexually selected traits is essential to understanding their evolution.

Here, we present the first detailed study of the inheritance and geographic distribution of a sex-limited discrete polymorphism in colour and size in males of the livebearing fish *Poecilia parae*. The morphs are sufficiently distinct that two of them were originally named as separate species: *P. vivipara parae* (Eigenmann, 1894) and *Acanthophacelus melanzonus* (Eigenmann, 1909). They were later classified as one species, *P. parae*, with three male morphs: parae, melanzona and the uncoloured immaculata (Rosen and Bailey, 1963). Within the melanzona morph, blue and red variants have previously been noted from Guyana (Liley, 1963) and blue and yellow variants from French Guiana (Keith *et al*, 2000).

We document five male morphs in *P. parae* and show that all five morphs can occur within a single population. We use field surveys to show that distribution patterns within and between populations suggest strong fitness effects. We then show that these morphs are probably under sexual selection by measuring variation in mating success in the wild, and the discrimination of males by females on the basis of colour in the laboratory. Finally, we use pedigree analyses to determine the genetic basis and linkage pattern of male colour morphs.

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Methods

Field surveys

P. parae occurs along the coast from Guyana to the mouth of the Amazon River in Brazil (Rosen and Bailey, 1963). To document the frequency of morphs and their geographic distribution, we searched for *P. parae* near the coast in ditches draining into rivers in Guyana and Suriname in four separate sets of field surveys (Guyana: June–August 1999, November 2000, January–February 2002; Suriname: May 2000). *P. parae* were only found in mud-bottomed ditches of a variety of sizes that served as sewerage outlets. We considered different drainage systems into a river as different sampling sites. Adult males and females were captured with dip nets or a $3\,m \times 1.5\,m$ seine net, and male colour patterns were scored. We noted potential predators, based on Winemiller *et al* (1990) and Endler (1978). At the end of each sampling period, fish were either released or taken into captivity.

Variation in morph mating success and investigation of genetic basis of colour morphs

Male and female *P. parae* were transported to Simon Fraser University, Canada from Georgetown, Guyana in 1999 and from Suriname in 2000. To determine the distribution of morphs among offspring of females that had mated in the wild, we isolated females in 5 l tanks for 3–5 months to allow them to produce offspring from field inseminations (poeciliids can store sperm for several months; Constantz, 1989). Surviving females were then assigned a mate. All offspring in a brood were transferred to a new tank after birth. Prior to sexual maturity (full gonopodial development), sons were separated from daughters to prevent uncontrolled inseminations. Male phenotypes were scored when colours developed, upon sexual maturity. Males were scored as 'immaculata' if they did not develop colour 2 months after sexual maturity. In no case did males initially scored as immaculata later develop colour. All fish were kept on a natural daylight schedule and fed with TetraMin fish flakes and brine shrimp nauplii.

To determine the genetic basis and linkage pattern of loci underlying colour morphs, we analysed pedigrees of males and females from Georgetown and their descendants. We assigned mates to virgin daughters and isolated and reared resulting offspring as above.

Dichotomous mate choice tests

Dichotomous choice tests were conducted in one of two identical glass aquaria in the morning from 22 June to 30 August 1999 in Georgetown at the laboratory of the guesthouse of the Smithsonian Research Institute. Each aquarium had four compartments: a central compartment ($23 \times 30\,cm^2$) and two end compartments ($11 \times 30\,cm^2$), one of which was divided to make two equal sized compartments ($11 \times 15\,cm^2$), one for each test male. The test female in the middle compartment was able to see both males at one end of the aquarium at the same time. At the compartment at the other end of the tank were two companion females who were housed there for days at a time and provided the test female an opportunity to school with other females rather than with only males. Males did not direct courtship towards these females.

Each aquarium contained 20 l of rainwater to a depth of 21 cm, and a tablespoon of sea salt. Rainwater was used to provide standard experimental conditions, as the colour of water collected from the field sites varied between days and between study sites. A layer of tan gravel lined the bottom of the tanks. Differences in the background exterior to the tanks were minimized by covering the glass with translucent waxed paper, except for the glass of the companion female compartment, through which the behaviour of the test female and males was observed. Lighting was provided by natural daylight and an Aquari-lux FL-20 full-spectrum aquarium light suspended 37 cm above the water surface and centred between the two tanks, which were placed 11 cm apart.

At the start of a choice test, a test female, a parae male and an equal-sized (within 1 mm in standard length) opponent male were placed into their respective compartments (see Figure 1 for photographs of males). The parae male was placed into the right compartment (with

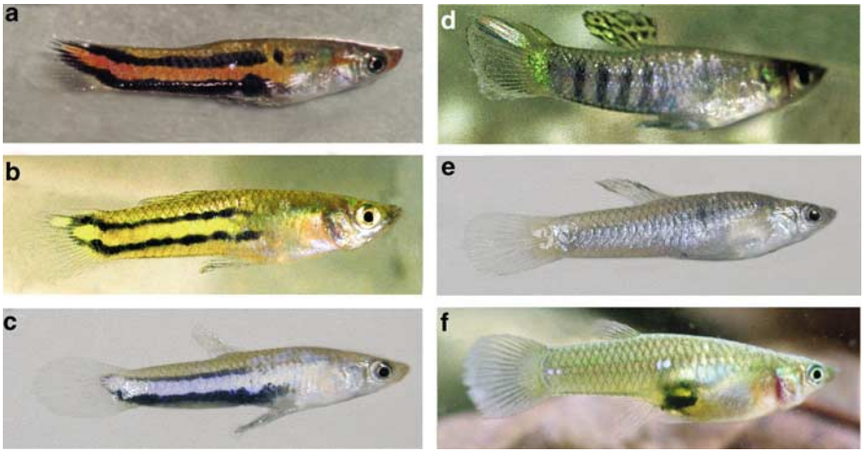

Figure 1 Male and female *Poecilia parae* from Guyana: (**a**) red melanzona, (**b**) yellow melanzona, (**c**) blue melanzona, (**d**) parae, (**e**) immaculata and (**f**) female. The immaculata male was photographed 12 months after capture as a sexually mature male.

respect to the observer) in half of the trials. Opaque and clear glass partitions divided the two male compartments, and also the male compartments and the test female compartment. After an acclimation period of 10 min, the opaque partitions were removed, and the behaviour of males and the female observed for 10 min. Removal of the opaque partition between males was important because it allowed the males to see each other, which increased male courtship (as in guppies *P. reticulata*; Farr, 1976). The opaque partitions were then replaced, and the males were switched between compartments. After another acclimation period, the opaque partitions were again removed and the fish were observed for an additional 10 min. The second observation period controlled for any side preferences of the female.

Fish behaviours were measured by the observer (AL) seated approximately 0.5 m away from the end of the tank housing the companion females. A computer with an event recorder program written by FB was used to record the number of seconds that the test female spent orientated toward each male against the glass of his compartment. The duration of attentiveness (defined as orientation to the female) was measured for each male. We also measured the numbers of dart displays, which were defined as extremely rapid movements to and fro (Liley, 1966). Dart-displays are the most common male display to precede copulation attempts in *P. parae* (Liley, 1966). Trials were considered successful if the female was orientated towards at least one male in each of the two observation periods, if the female swam calmly in the tank, and if both males showed interest in the female. Females and males from a successful trial were not used again. In total, 97 trials were conducted, of which 56 were successful. Of 56 females, 32 were tested within 48 h of parturition, 13 were tested later in the reproductive cycle and the remaining 11 females did not give birth in the laboratory. These trials tested female preference between 56 different parae males paired with 47 melanzona and nine immaculata males.

Results

Five male morphs

The five male morphs are shown in Figure 1. Three morphs, the yellow, red and blue, are of the melanzona type, with double horizontal black stripes along the length of the body. For simplicity, these morphs will be called hereafter the yellow, red and blue morphs. The parae morph has a colourful tail stripe and vertical bars which are facultatively expressed. The fifth morph, the immaculata, lacks stripes and thus has juvenile colouration. This colouration is developmentally stable – 13 wild-caught sexually mature immaculata did not alter colour pattern during 6 months of captivity (see also the caption of Figure 1). The morphs varied in standard length (Table 1), as immaculata males were significantly smaller than the other four types (Tukey test, all comparisons with immaculata males $P<0.05$, all others not significant).

Field surveys

The parae morph was more abundant than red, blue or yellow males at all nine sites and in all years (Table 2), including samples from 1954 and 1956 (Liley, 1963). In 11/12 surveys, blue was next most abundant, leaving red and yellow as the least abundant morphs. Within this broad trend, there was significant heterogeneity in morph frequencies between years at one of two sites that were surveyed twice (Demerara West 3 ($G=11.79$, $df=2$, $P<0.005$) *vs* Demerara East 1 ($G=5.05$, $df=3$, $P>0.1$)), and across all sites ($G=53.29$, $df=24$, $P<0.001$, using the largest survey at Demerara West 3 and Demerara East 1). Notably, at four sites red males were not detected. We tested whether the distribution of morph frequencies varied geographically using a Mantel test. For sites that had been surveyed more than once, we used only the largest survey. There was no correlation ($r=0.008$, $P=0.33$) between the matrix of geographical coordinates of sampling sites and the matrix of frequencies of parae, red, blue and yellow males. Thus, we found no evidence of a systematic effect of geographical location on morph frequencies.

Table 1 Standard length of male morphs plus ANOVA test statistic

Morph	N	Standard length		*F*	*df*	P
		Mean	*SD*			
Parae	200	19.2	1.4	13.80	4332	0.001
Blue	37	18.9	1.2			
Red	37	19.1	1.1			
Yellow	30	18.4	1.4			
Immaculata	33	17.4	1.1			

We could not include data for immaculata males, as these males could not be distinguished in the field from nearly mature juveniles of other morphs, which had yet to develop colour. Thus, the frequency of uncoloured males scored in 2002 surveys overestimated the frequencies of immaculata, as some uncoloured males are immature parae, red, blue or yellow males. These data do however place an upper boundary on estimates of immaculata frequency for these samples, at up to 53.4% (Table 2) of all sexually mature males.

The predator communities probably varied among sites. At Demerara River West sites 3 and 4, water depth was shallow, ranging from 5–40 cm and the only potential predators observed were *Hemigrammus* spp. (up to 7 cm long) and *Rivulus* spp. (up to 5.5 cm long). At all sites on the East bank of the Demerara River, and site 2 on the West Bank, trenches were larger and water depth was up to 1.5 m. These were local fishing sites for the cichlids *Oreochromis mossambicus* and *Crenicichla* spp.

Variation in morph mating success

Nine wild-caught females from Georgetown and six from Suriname produced sons in the laboratory in Canada from field inseminations. Eight of the nine females from Georgetown produced only parae sons ($N=19$), while one produced both parae ($N=4$) and immaculata sons ($N=6$). The six females from Suriname produced only parae sons ($N=8$). As a conservative test of the hypothesis that parae males have higher mating success relative to their frequency in the population (relative to the melanzona morphs), we compared the observed frequencies of parae litters with an expectation based on a proportion of 69% (this proportion is the

Table 2 Counts of colour morphs by site and year

Site	*Coordinates*		*Sampling year*	*Parae*		*Blue*		*Yellow*		*Red*		*Uncoloured*
	N	W		N	*(%)*	N	*(%)*	N	*(%)*	N	*(%)*	N
Demerara R. East 1	6°49.427	58°09.550	1999	200	(69.0)	41	(14.1)	23	(8.0)	26	(8.9)	
			2000	27	(84.3)	1	(3.1)	2	(6.3)	2	(6.3)	
Demerara R. East 2	6°48.315	58°09.076	1999	36	(61.0)	15	(25.4)	6	(10.2)	2	(3.4)	
			1954[a]	5	(55.6)							
			1956[a]	7	(53.8)							
Demerara R. East 3	6°48.036	58°09.069	2002	112	(43.6)	83	(32.3)	17	(6.6)	45	(17.5)	102
Demerara R. West 1	6°47.797	58°11.112	2002	111	(66.9)	30	(18.1)	5	(3.0)	20	(12.0)	136
Demerara R. West 2	6°47.148	58°11.497	2002	33	(60.0)	18	(30.5)	8	(13.5)	0	(0)	20
Demerara R. West 3	6°42.722	58°12.452	2000	77	(93.9)	4	(4.9)	1	(1.2)	0	(0)	
			2002	101	(77.1)	22	(16.8)	8	(6.1)	0	(0)	150
Demerara R. West 4	6°41.472	58°11.858	2002	714	(70.0)	234	(22.9)	66	(6.5)	6	(0.6)	915
Berbice R. West	6°16.347	58°32.511	2002	108	(74.0)	26	(17.8)	12	(8.2)	0	(0)	155
Suriname R. East	6°53.075	58°05.375	2000	27	(56.3)	11	(22.9)	10	(20.8)	0	(0)	

[a]Data from Liley (1963), who compared the frequency of parae *vs* all melanzona males.
Percentage composition of parae, blue, yellow and red in 2002 was calculated without the inclusion of uncoloured males to facilitate comparisons with surveys from earlier years.

highest of the three proportions of parae:melanzona males in the sites from which these females were collected – Demerara East 1 and 2, and Suriname River West). Parae males had significantly higher mating success than expected from their proportion of 0.69 in the population ($G_1 = 11.11$, $P = 0.001$).

Inheritance of colour morphs

Controlled matings showed a simple genetic basis to the colour morphs. In 14/14 cases, laboratory-reared females that were mated as virgins bore sons displaying the paternal phenotype (Table 3). The exact probability that all 23 sons matched their paternal phenotype by chance alone is $(1/5)^{23}$. Further evidence of paternal inheritance comes from females that produced sons of different phenotypes following remating. Three field-inseminated females that had parae sons in the laboratory were later remated to a red, blue or yellow male. These females subsequently produced no more parae sons, but sons that matched the morph of the male used in the remating. Another female had immaculata sons followed by a brood of red sons, after remating to a red male. These patterns of inheritance suggest that the alleles determining male colour patterns are linked to the Y-chromosome.

Pedigrees for descendants of three wild-caught females (including two of the four remated females just discussed) further show that colour patterns are inherited through the sire (Figure 2), with no apparent influence of maternal genotype. The pedigree of the descendants of female A rules out X-linkage of the alleles for colour. One obvious autosomal model, assuming that the parae morph is recessive, can be rejected at $P < 0.05$ (this model is explained in the caption to Figure 2). Thus, the pattern of inheritance is completely consistent with linkage to the Y-chromosome, but inconsistent with X-chromosome or autosomal linkage of colour-determining alleles.

Female discrimination of colour morphs

As the parae morph was most successful in terms of frequency in the wild and in mating success, we hypothesized that females would prefer the parae morph to all others. Thus, we designed female choice tests to compare female preferences for the parae morph against each of the other morphs, while controlling for differences in body size. Females spent less time with parae than with yellow males in 11/13 trials and less time with parae than red males in 15/21 trials (binomial tests, one-tailed, $P < 0.04$). Immaculata were less attractive than equal-sized parae males, as females spent more time with parae than immaculata in 8/9 trials (binomial test, one-tailed, $P < 0.02$). Blue melanzona males were preferred to parae males in 6/13 trials (binomial test, one-tailed, $P = 0.71$). It is not clear, however, that females showed no preference, as the power of the test was low (power = 0.27 for $N = 13$, effect size = 0.25, alpha = 0.05, one-tailed).

We tested if males differed in their courtship behaviour, as this might influence female preferences. However, male colour morphs did not differ in their courtship behaviour during trials. Immaculata, parae, red, yellow and blue males performed similarly in terms of attentiveness time and number of darts performed (Table 4).

Table 3 Offspring phenotype *vs* paternal phenotype from 14 crosses, scored per female with total number of sons produced in parentheses

Son's phenotype	*Paternal phenotype*[a]			
	Red	*Yellow*	*Parae*	*Immaculata*
Red	4 (7)			
Yellow		2 (4)		
Parae			6 (8)	
Immaculata				2 (4)

[a]No blue males produced mature male progeny in this experiment.

Discussion

P. parae has five distinct male-specific morphs that differ in colour pattern and body size. Male colour morphs have a genetic basis that is consistent with a simple model of five alternative Y-linked alleles. In well-studied

systems of male-limited colour morphs, allelomorphs underlie variation in male colour pattern (Angus, 1989; Shuster and Wade, 1991; Sinervo, 2001; Lindholm and Breden, 2002), which is consistent with our results. In livebearing fishes (Poeciliidae), 12 species other than *P. parae* have Y-linked alleles that cause variation in male colour or body size (Lindholm and Breden, 2002). In contrast to mammals, Y-chromosomes are typically not highly degenerate in poeciliids, which presumably facilitates Y-linkage of functional genes.

We have demonstrated that the parae morph is by far the most abundant morph at all sites sampled and over time periods greater than 40 years, and that parae males enjoy greater mating success in the wild than would be expected based on their frequency in the population. Furthermore, we have shown that females can discriminate between males of different morphs in a way that is consistent with mate choice. Thus, there appear to be strong fitness effects associated with colour morph, and colour morphs are likely to be under sexual selection.

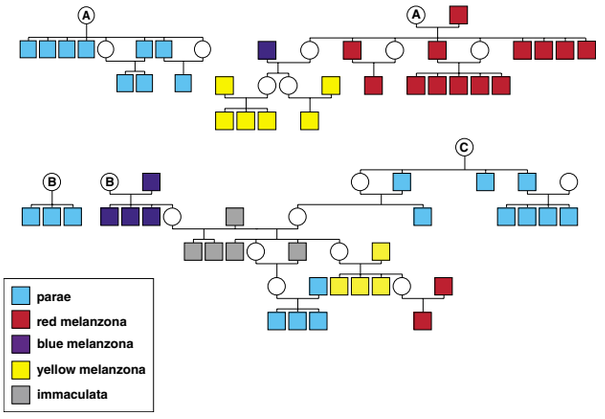

Figure 2 Pedigrees for offspring of wild-caught females A, B and C. All three produced offspring from field inseminations, but A and B were later remated to males of known phenotype, and are therefore represented twice. Daughters that produced no male descendants were omitted. This pedigree can be used to reject X-linkage: under the X-linked model, female A must have X-linked alleles for the parae and red morphs. Her daughter mated to the blue morph would therefore have no X-linked alleles for the yellow morph, and yet she produces yellow males. This pedigree can also be used to calculate exact probabilities for the autosomal model, under various dominance relations among the alleles and assumptions about population gene and genotype frequencies. One obvious model would be to assume that the parae morph is recessive to all other morphs, thus making the change from parae male offspring to male offspring with the new father's phenotype most likely. Assuming that unknown genotypes are homozygous for the autosomal parae allele (this morph has the highest frequency in all natural populations) and that each female is an independent test of the autosomal model (ie, using Fisher's combined probabilities test over all females; Sokal and Rohlf, 1969), the probability of perfect association between paternal phenotype and male offspring phenotype is less than 0.05.

The high mating success of parae males might reflect a female preference for mating with males of that morph. However, dichotomous choice tests provide no evidence for this and instead indicate that females discriminate against parae in favour of yellow and red males. Another study of preference in this species has also observed that the yellow and red melanzona morphs are preferred by females in choice tests (Bourne *et al*, 2003). In our present study, females only preferred to associate with parae when given a choice between parae and the uncoloured immaculata. These results suggest that the high mating success achieved by parae is not simply due to female choice, but may be due to other sexually selected advantages such as superior ability in overt male–male contests, in sperm competition, or in obtaining sneaky copulations. There is as yet no evidence for this, as we found no differences in male courtship behaviour between morphs in our female choice tests. However, Magurran (1998) has argued that such male-driven factors can overcome female preference in the guppy, which is closely-related to *P. parae* (Breden *et al*. 1999).

Female preferences for associating with red and yellow males suggest that negative frequency-dependent female mate choice is a possible mechanism for the maintenance of these rarer morphs. Female side-blotched lizards *Uta stansburiana* maximise their fitness by mating with rare males in low-density years (Alonzo and Sinervo 2001). In the guppy, rare or novel males enjoy a mating advantage (Farr, 1977; Hughes *et al*, 1999). As yet, we have no evidence of differential mating success in favour of red and yellow males in *P. parae*. Other selective pressures and processes might act in the opposite direction, to reduce the frequency of these rare but brightly coloured morphs. They may experience higher rates of predation, as is believed to be the case in guppies (Haskins *et al*, 1961; Endler, 1983; Godin and McDonough, 2003). Differential predation might therefore reduce the numbers of red and yellow males relative to parae and immaculata males. Although we did not measure predation rates, we found the highest frequencies of red males in large ditches supporting large predatory fish, suggesting that there is not a simple relationship between predation risk and relative frequency of brightly coloured males.

Table 4 Comparison of male courtship

Morph	N	*Attentiveness (s)*					*Dart displays*				
		Mean	*SD*	χ^2	*df*	P[a]	*Mean*	*SD*	χ^2	*df*	P[a]
Parae	56	801.6	298.4	3.65	4	0.46	0.85	2.55	3.96	4	0.41
Blue	13	916.8	336.8				1.15	2.27			
Red	21	787.5	301.2				2.55	6.07			
Yellow	13	909.9	209.6				0.23	0.44			
Immaculata	9	784.7	222.1				1.44	2.01			

[a]Based on Kruskall–Wallis rank sum test statistic.

Very low frequencies of any morph would render it susceptible to within-population extinction by demographic stochasticity (Calsbeek *et al*, 2002). Y-linkage of morph-determining alleles would exacerbate this process by reducing the effective population size of these alleles. This is a plausible explanation for the absence of red males from some populations, as we found no evidence of systematic geographic variation in morph frequencies among our study sites. There may, however, be geographic variation over a broader scale, as the red morph has not been reported from French Guiana despite extensive surveys (Keith *et al*, 2000; P-Y Le Bail, pers. comm.).

Male-beneficial, sexually antagonistic alleles are thought to preferentially accumulate on the Y-chromosome in male heterogametic species, because there they will not be expressed in females (Fisher, 1931; Rice, 1987). Despite strong theoretic (Rice, 1984, 1987) and experimental (Rice, 1992, 1994) evidence that sexually antagonistic genes should accumulate on Y-chromosomes, there are few compelling examples in natural systems. In *P. parae*, the relationship between male morph and sexual selection suggests that the genes underlying male morph may be sexually antagonistic. There is evidence from the guppy that genes determining attractive male colour patterns are associated with increased predation risk (Endler, 1983) and nonpredatory mortality (Brooks, 2000), which suggests that were such genes to be expressed in females they would confer a net decrement in fitness (ie they probably are sexually antagonistic). The Y-linkage of male colour morph genes in *P. parae* supports the hypothesis that such genes should be linked to the heterogametic chromosome.

P. parae exhibits one of the most complex sex-limited discrete polymorphisms known. Some closely related species are characterized by additional discrete polymorphisms, such as that found in *P. picta*, while the well-studied guppy, *P. reticulata*, exhibits nearly continuous variation. Thus, this clade presents a unique opportunity to study the role of sexual selection in the maintenance of male colour polymorphism.

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References

Alonzo SH, Sinervo B (2001). Mate choice games, context-dependent good genes, and genetic cycles in the side-blotched lizard, *Uta stansburiana*. *Behav Ecol Sociobiol* **49**: 176–186.

Angus RA (1989). A genetic overview of poeciliid fishes. In: Meffe GK, Snelson Jr FF (eds) *Ecology and Evolution of Livebearing Fishes (Poeciliidae)*. Prentice-Hall: Englewood Cliffs, NJ. pp 51–68.

Bourne GR, Breden F, Allen TC (2003). Females prefer carotenoid males as mates in the pentamorphic livebearing fish, *Poecilia parae*. *Natur Wissenschaften* **90**: 402–405.

Breden F, Ptacek M, Rashed M, Taphorn D, Augusto de Figueiredo C (1999). Molecular phylogeny of the live-bearing fish genus *Poecilia* (Cyprinodontiformes: Poeciliidae). *Mol Phylogenet Evol* **12**: 95–104.

Brooks R (2000). Negative genetic correlation between male sexual attractiveness and survival. *Nature* **406**: 67–70.

Calsbeek R, Alonzo SH, Zamudio K, Sinervo B (2002). Sexual selection and alternative mating behaviours generate demographic stochasticity in small populations. *Proc Roy Soc London B* **269**: 157–164.

Clarke B (1962). Balanced polymorphism and the diversity of sympatric species. In: Nichols D (ed) *Taxonomy and Geography.* Systematics Association: Oxford. pp 47–70.

Constantz GD (1989). Reproductive biology of poeciliid fishes. In: Meffe GK, Snelson Jr. FF (eds) *Ecology and Evolution of Livebearing Fishes (Poeciliidae)*. Prentice-Hall: Englewood Cliffs, NJ. pp 33–49.

Eigenmann CH (1894). Notes on some South American fishes. *Ann N Y Acad Sci* **7**: 625–637.

Eigenmann CH (1909). Reports on the expedition to British Guiana of the Indiana University and the Carnegie Museum, 1908. Some new genera and species of fishes from British Guiana. *Ann Carnegie Mus* **6**: 4–54.

Endler JA (1978). A predator's view of animal color patterns. *Evol Biol* **11**: 319–364.

Endler JA (1983). Natural and sexual selection on color patterns in poeciliid fishes. *Env Biol Fish* **9**: 173–190.

Farr JA (1976). Social facilitation of male sexual behavior, intrasexual competition, and sexual selection in the guppy, *Poecilia reticulata* (Pisces: Poeciliidae). *Evolution* **30**: 707–717.

Farr JA (1977). Male rarity or novelty, female choice behavior, and sexual selection in the guppy, *Poecilia reticulata* Peters (Pisces: Poeciliidae). *Evolution* **31**: 162–168.

Fisher RA (1931). The evolution of dominance. *Biol Rev* **6**: 345–368.

Godin, J-GJ, McDonough HE (2003). Predator preference for brightly colored males in the guppy: a viability cost for a sexually selected trait. *Behav Ecol* **14**: 194–200.

Haskins CP, Haskins EF, McLaughlin JJA, Hewitt RE (1961). Polymorphism and population structure in *Lebistes reticulatus*, an ecological study. In: Blair, WF (ed) *Vertebrate Speciation*. University of Texas Press: Austin. pp 320–395.

Hughes KA, Du L, Rodd FH, Reznick DN (1999). Familiarity leads to female mate preference for novel males in the guppy, *Poecilia reticulata*. *Anim Behav* **58**: 907–916.

Keith P, Le Bail P-Y, Planquette P (2000). *Atlas des Poissons d'Eau Douce de Guyana*, tome 2 fasicule 1. Muséum National d'Histoire Naturelle: Paris.

Kondrashov AS, Kondrashov FA (1999). Interactions among quantitative traits in the course of sympatric speciation. *Nature* **400**: 351–354.
Lande R (1981). Models of speciation by sexual selection on polygenic traits. *Proc Natl Acad Sci USA* **78**: 3721–3725.
Lande R, Wilkinson GS (1999). Models of sex-ratio meiotic drive and sexual selection in stalk-eyed flies. *Genet Res* **74**: 245–253.
Liley NR (1963). Reproductive Isolation in Some Sympatric Species of Fishes. DPhil Thesis, Oxford.
Liley NR (1966). Ethological isolating mechanisms in four sympatric species of poeciliid fishes. *Behav Suppl* **13**: 1–197.
Lindholm AK, Breden F (2002). Sex chromosomes and sexual selection in poeciliid fishes. *Am Nat* **160**: S214–S224.
Magurran A (1998). Population differentiation without speciation. *Phil Trans Roy Soc London B* **353**: 275–286.
Reinhold K (1998). Sex linkage among genes controlling sexually selected traits. *Behav Ecol Sociobiol* **44**: 1–7.
Ritchie MG, Phillips SDF (1998). The genetics of sexual isolation. In: Howard DJ, Berlocher SH (eds) *Endless Forms*. Oxford University Press: Oxford. pp 291–308.
Rice WR (1984). Sex chromosomes and the evolution of sexual dimorphism. *Evolution* **38**: 735–742.
Rice WR (1987). Genetic hitchiking and the evolution of reduced genetic activity of the Y sex chromosome. *Genetics* **116**: 161–167.
Rice WR (1992). Sexually antagonistic genes: experimental evidence. *Science* **256**: 1436–1439.
Rice WR (1994). Degeneration of a nonrecombining chromosome. *Science* **263**: 230–232.
Rosen DE, Bailey RM (1963). The poeciliid fishes (Cyprinodontiformes), their structure, zoogeography, and systematics. *Bull Am Mus Nat Hist* **126**: 3–126.
Shuster SM, Wade MJ (1991). Equal mating success among male reproductive strategies in a marine isopod. *Nature* **350**: 608–610.
Sokal RR, Rohlf FJ (1969). *Biometry*. Freeman: San Francisco.
Sinervo B (2001). Runaway social games, genetic cycles driven by alternative male and female strategies, and the origin of morphs. *Genetica* **112–113**: 417–434.
Sinervo B, Svensson E (2002). Correlational selection and the evolution of genomic architecture. *Heredity* **89**: 329–338.
Winemiller KO, Leslie M, Roche R (1990). Phenotypic variation in male guppies from natural inland populations: an additional test of Haskins' sexual selection/predation hypothesis. *Environ Biol Fish* **29**: 179–191.

Chapter 2

Sex chromosomes and sexual selection in poeciliid fishes

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Sex Chromosomes and Sexual Selection in Poeciliid Fishes

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ABSTRACT: We propose that the evolution of female preferences can be strongly influenced by linkage of attractive male traits to the Y chromosome and female preferences to the X chromosome in male heterogametic species. Such linkage patterns are predicted by models of the evolution of sexually antagonistic genes. Subsequent recombination of attractive male characters from the Y to the X would create physical linkage between attractive male trait and preference. A literature survey shows that Y linkage of potentially sexually antagonistic traits is common in poeciliid fishes and other species with sex chromosomes that are not well differentiated, but may also occur in taxa with degenerate Y chromosomes. In the guppy, attractive male traits are primarily Y and X linked; a literature review of the inheritance of sex-limited attractive male characters suggests that 16 are Y linked, 24 recombine between the X and Y, two are X linked, and two are autosomal. Crosses and backcrosses between high female preference (Endler's live-bearers) and low female preference (Rio San Miguel) guppy populations show that this character has a strong additive genetic component and that it will be possible to investigate the physical linkage of male and female sexually selected characters in this species through mapping studies.

Keywords: sexual selection, sex chromosomes, Y chromosome, Poeciliidae, *Poecilia reticulata.*

To what extent the genetic architecture of behavior constrains its evolution remains highly controversial. Genomic data and molecular genetic techniques recently developed in model genetic organisms and applied to model behavioral systems will help answer this question. One area in which these approaches may be particularly fruitful will be in understanding how linkage to sex chromosomes affects the process of sexual selection. Fisher noted two ways in which the linkage of traits could affect the dynamics of sexual selection. First, the conditions for the evolution of a sexually antagonistic trait, a trait that benefits one sex but is detrimental to the other, depend on whether the genes controlling that trait are linked to the sex chromosomes or to autosomes (Fisher 1931). Second, genetic correlations between sexual preferences for attractive traits and the traits themselves can lead to rapid coevolution of these characters (Fisher 1958); such genetic correlations are more easily maintained when there is physical linkage between genes controlling the traits. Determining the interplay between sex chromosomes and the linkage of genes for attractive male traits and female preferences has the potential to answer several long-standing questions in sexual selection.

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Effect of Linkage to Sex Chromosomes on the Dynamics of Sexually Selected Traits

Linkage of Sexually Antagonistic Genes to Heterogametic Sex Chromosomes

Evolutionary theory predicts that sexually antagonistic genes that benefit the heterogametic sex are more likely to increase when linked to the sex-determining locus (Fisher 1931; Bull 1983; Rice 1987*a*; see table 1 for definition of terms). This prediction is a consequence of the fact that sexually antagonistic genes that are linked to the sex-determining locus are rarely expressed in the homogametic sex, providing little or no opportunity for selection against these genes. However, the accumulation of sexually antagonistic genes in linkage with the sex-determining locus favors a reduction in recombination between the sex chromosomes (Rice 1987*a*). This is predicted to lead to the decay of nonrecombining regions, since any genetic load that accumulates through Muller's ratchet, genetic hitchhiking, or other mechanisms cannot be purged by recombination (Charlesworth 1978; Rice 1987*b*). This degeneration in turn may make it less likely that functional genes will be linked to the heterogametic sex chromosome.

We conducted a survey of the literature to estimate the frequency with which phenotypic traits that are potentially sexually antagonistic are presently linked to the heterogametic sex chromosome. We ignored characters involved in sexual differentiation, such as genes controlling spermatogenesis in humans (Lahn and Page 1997) or eggshell

Table 1: Definitions used in sexual selection and sex chromosome studies

Term	Definition
Sex chromosomes	A pair of chromosomes with a locus that determines sex
Heteromorphic sex chromosomes	A pair of sex chromosomes, one of which harbors few functional genes, is usually reduced in size, and exhibits reduced recombination with the other sex chromosome
Heterogametic sex	Sex that is determined by a dominant allele at the sex-determining locus; this is often denoted as XY for male heterogamety and ZW for female heterogamety
Heterogametic sex chromosome	The chromosome that harbors the dominant sex-determining allele (Y in XY systems, W in ZW systems)
Pseudoautosomal region	Region of sex chromosomes with functional genes and recombination rates similar to autosomes
Sex-limited gene	Locus whose alleles are expressed only in one sex; this locus is not necessarily located on the sex chromosomes
Sex-linked gene	Locus physically linked to the sex chromosomes; this locus can be in a recombining (pseudoautosomal) or nonrecombining region
Sexually antagonistic gene	Gene whose expression is beneficial to one sex but detrimental to the other sex

patterning in birds (Gosler et al. 2000), because these can only be expressed in a functional member of one sex and therefore cannot be sexually antagonistic. The results (table 2) show that most cases are found in fishes and especially in poeciliids, a family of live-bearing fishes that varies within and between species in sex-determination system and sex chromosome structure (Kallman 1975; Black and Howell 1979; Angus 1989). Sex-limited color patterns in poeciliids, such as those referred to in table 2, are considered sexually antagonistic traits (Fisher 1931; Endler 1980; Bull 1983).

The large number of cases of Y linkage in fishes is probably related to the fact that teleost fishes are mostly characterized by morphologically similar (homomorphic) sex chromosomes (Ohno 1974), so the X and Y chromosomes are equally represented in the genome. However, Y linkage also occurs in chrysomelid beetles, mice, and humans (table 2), which have heteromorphic sex chromosomes with a degenerate Y (Smith and Virkki 1978; Segarra and Petitpierre 1990; Graves 1995). Several of the cases of linkage to the heterogametic sex chromosome in table 2 involve poorly studied species, and this implies that there may be many more undiscovered cases of such linkage. Furthermore, taxa that presently have few genes linked to the heterogametic sex chromosome, such as birds and mammals, presumably passed through a period in their evolution during which many genes were linked to the evolving sex chromosomes (Bull 1983). This linkage could have been a driving force for the decay of the Y chromosome (Rice 1987*b*) and also may have affected the evolution of sexually selected traits, even though these genes are not presently linked to the heterogametic sex chromosome. Comparative studies of groups exhibiting homomorphic and heteromorphic sex chromosomes could determine the effect of the evolution of differentiated sex chromosomes on the evolutionary dynamics of sexually selected traits.

Sex Chromosomes and Linkage between Female Preference and Attractive Male Characters

Traits linked to the heterogametic sex chromosome are often male secondary sex characters important in sexual selection (table 2). Explaining the evolution of female preferences for these characters is one of the most difficult challenges in sexual selection, and we propose that patterns of linkage to sex chromosomes could greatly affect this evolution.

Models for the evolution of female preference can be characterized according to whether there is direct or indirect selection on preference. Indirect models depend on a genetic correlation between female preference and other characters undergoing positive direct selection, such as "good genes" that confer overall higher fitness due to natural selection in offspring or the attractive male characters themselves. Genetic correlations may be caused by either pleiotropy or linkage disequilibrium. It is commonly assumed that the genetic correlation in sexual selection models is due to linkage disequilibrium between genes on different chromosomes caused by nonrandom mating (Lande 1981). Indirect models have been criticized on the basis that linkage disequilibrium caused by nonrandom mating would be difficult to maintain for any length of time because of genetic drift and recombination in finite populations (Nichols and Butlin 1989) and that the force of indirect selection caused by this linkage would be weak (Barton and Turelli 1991; Kirkpatrick and Barton 1997).

Predominant linkage of attractive male traits to the heterogametic sex chromosome may have important implications for these indirect models. Genetic correlations between female preferences and attractive male traits that are exclusively Y linked cannot occur through pleiotropy or physical linkage, since daughters will not inherit Y-linked genes (in male heterogametic taxa). Linkage dis-

Table 2: Phenotypic traits unrelated to sexual differentiation that are linked to the Y or W (heterogametic) chromosome

Family and species	Trait	Heterogametic sex	Reference
Chrysomelidae:			
Gonioctena variabilis	Pigmentation	Male	de Zulueta 1925; Galán 1931
Phyllotreta nemorum	Survival on host plant	Male	Segarra and Petitpierre 1990; Nielsen 1997
Cichlidae:			
Neochromis omnicaeruleus	Pigmentation	Male or female[a]	Seehausen et al. 1999
Cyprinodontidae:			
Oryzias latipes	Pigmentation	Male	Matsuda et al. 1998; Wada et al. 1998
Hominidae:			
Human	Height, tooth growth	Male	Alvesalo 1997; Kirsch et al. 2000
Muridae:			
Mus musculus	Aggression	Male	Selmanoff et al. 1975; Sluyter et al. 1994
Papilionidae:			
Papilio glaucus	Pigmentation	Female	Scriber et al. 1996
Poeciliidae:			
Gambusia holbrooki	Pigmentation	Male	Black and Howell 1979; Angus 1989
Limia perugiae	Size	Male	Erbelding-Denk et al. 1994
Poecilia latipinna	Size	Male	Travis 1994
Poecilia parae	Pigmentation	Male	A. Lindholm and F. Breden, unpublished data
Poecilia reticulata	Pigmentation, fin shape and size, courtship, attractiveness	Male	Winge 1927; Farr 1983; Brooks 2000; Brooks and Endler 2001
Xiphophorus andersi	Size	Male	Kallman 1989
Xiphophorus maculatus	Pigmentation, size	Male or female[a]	Kallman 1970; Kallman and Borkoski 1978
Xiphophorus milleri	Pigmentation, size	Male	Kallman and Borowsky 1972
Xiphophorus montezumae	Pigmentation, size	Male	Kallman 1983
Xiphophorus multilineatus[b]	Pigmentation, bar suppressor, size, courtship	Male	Zimmerer and Kallman 1988, 1989; Kallman 1989
Xiphophorus nigrensis[b]	Pigmentation, size	Male	Zander 1968; Kallman 1989
Xiphophorus pygmaeus	Pigmentation, size	Male	Kallman 1989
Xiphophorus variatus	Pigmentation, size	Male	Borowsky 1984, 1987

[a] Multiallelic system.
[b] Sensu Rauchenberger et al. 1990.

equilibrium between genes on different chromosomes is thus the only mechanism that could account for a genetic correlation between genes for female preference and genes for male attractiveness that are exclusively Y linked. However, we propose that some recombination between X and Y chromosomes could produce physical linkage between preference and trait when female preference genes are also linked to the X chromosome. X linkage of preference genes could occur by chance; that is, a certain proportion of new mutants for female preference would be expected to be X linked. However, if female preferences are sexually antagonistic and alleles that determine them are dominant, theory predicts that these alleles would be preferentially linked to the X chromosome, because the X chromosome spends two-thirds of its time in the homogametic sex (Rice 1984). Female preferences might be expected to be sexually antagonistic because females obtain whatever benefit is driving the evolution of female preference, but males might only pay the costs (e.g., association with conspicuous conspecifics in an environment with a high risk of predation).

If alleles influencing female preferences are linked to the X chromosome, and an allele for female preference on the X crosses over to the Y chromosome, it is no longer exposed to selection for choosiness. However, if many attractive male traits are linked to the heterogametic sex chromosome in a region that occasionally recombines with the X (as observed in guppies; Winge 1934), and if an allele for an attractive male trait recombines from the Y to the X, then alleles for the attractive trait and alleles for the preference will be physically linked. Reduced recombination between the X and Y chromosomes in such regions implies that alleles for the attractive male traits

would not often recombine from the Y to the X, but when they do, they would be closely linked to alleles for preference on the X chromosome. This physical linkage could greatly reduce recombination between genes for male trait and female preference and facilitate the evolution of female preferences.

If indeed female preferences are preferentially linked to the X chromosome, then many factors would determine the potential for the establishment and maintenance of genetic correlations caused by this linkage. These would include recombination rates between the X and Y and between homologous X chromosomes within females, selection on associated attractive characters and preferences, and the pattern of dosage compensation, which affects selection on antagonistic genes (Charlesworth et al. 1987). The interplay between linkage to sex chromosomes and the dynamics of sexual selection is only beginning to be modeled (Lande and Wilkinson 1999), but in general, physical linkage should enhance genetic correlations between preference and attractive character.

In addition to further theoretical work, it is critical to answer several empirical questions concerning linkage patterns of alleles controlling sexually selected characters. What are the patterns of linkage and recombination between attractive male characters in natural populations? Are female preference genes preferentially associated with the sex chromosomes? If so, what are the rates of recombination between genes for preference and genes for attractive male characters linked to these sex chromosomes?

The guppy, *Poecilia reticulata*, is an ideal system with which to examine these questions; it possesses sex chromosomes with many X- and Y-linked male secondary sexual characters (table 3), has male heterogamety, and exhibits genetically determined variation for female preference for these characters. Another poeciliid, *Xiphophorus maculatus*, shows male or female heterogamety and also has X- and Y-linked alleles for pigmentation patterns and body size (Kallman 1975), but genetic variation in female preference for these traits has not yet been demonstrated. This variation in female preference is necessary in order to identify genes underlying preference and to estimate linkage with other genes. The potential for mapping genes controlling variation in attractive male characters and mating preferences and determining their linkage patterns in guppies is greatly enhanced by a genomics project in the closely related poeciliid, *Xiphophorus helleri*. This genomics project is based on a genetic model for melanoma (Kazianis et al. 1998; http://www.xiphophorus.org).

Linkage of Attractive Male Characters to Sex Chromosomes in Guppies

Guppy males exhibit many elaborate secondary sexual characters, and guppy populations exhibit extreme polymorphism for these characters. Several have been shown to be attractive to females: conspicuous coloration, especially bright orange and black spots, large caudal fins, large body size, and high courtship display rate (Farr 1980; Bischoff et al. 1985; Reynolds and Gross 1992; Nicoletto 1993; Endler and Houde 1995; Brooks and Endler 2001). A survey of the literature on the inheritance of these attractive male traits shows that color patterns, caudal fin size and shape, courtship rates, and a composite measure of attractiveness are primarily sex linked in guppies (table 3). An exception is body size, which shows high heritability but has not been shown to be sex linked (Reynolds and Gross 1992; Yamanaka et al. 1995; Brooks and Endler 2001). Only one X-linked gene has been found in guppies that is unlikely to be sexually selected: a low-temperature-resistance gene that is expressed in both males and females (Fujio et al. 1990). Both quantitative genetic and pedigree analyses indicate that most of the attractive male traits are not exclusively Y-linked (table 3; see Winge 1927 or Kirpichnikov 1981 for drawings of many of the named combinations of color patterns and fin morphologies). Many of these traits recombine between the X and Y chromosomes, revealing the homology between guppy sex chromosomes.

It has recently been shown that there is some cytological and molecular differentiation between the X and Y chromosomes in the guppy (Traut and Winking 2001). Only one-half of the Y chromosome pairs with homologous regions of the X in synaptonemal complexes. Furthermore, the orientation of the chromosomes allowed for recombination in only two of 49 synaptonemal complexes observed; this suggests that recombination is also greatly reduced in the pairing, homologous region. Comparative genomic hybridization indicated that a large part of the nonpairing region of the Y chromosome comprises male-specific repetitive DNA (Traut and Winking 2001) and that there is structural variation among Y chromosomes in this region. This agrees with results from an in situ hybridization study showing that Y chromosomes, but not X chromosomes, of some domesticated guppies carry large numbers of simple repetitive sequences (Nanda et al. 1990). However, these male-specific repeats were not observed in recent descendants of wild guppies (Hornaday et al. 1994). Degeneration of the Y chromosome is supported by the observation that inheritance of Y chromosomes bearing alleles for attractive male traits leads to increased mortality (Brooks 2000). The buildup of simple repetitive sequences and deleterious mutations on Y chromosomes that produce male guppies highly attractive to females would provide a mechanism for the result that more attractive males produce sons of lower viability.

This picture of the sex chromosomes concurs with that inferred from pedigree analyses. Some gene complexes

Table 3: Linkage of sexually selected male traits in guppies

		Crossover frequency		
	N	X to Y % (*N*)	Y to X % (*N*)	Reference
Y linked:[a]				
Maculatus-red	3,841			Schmidt 1920; Winge 1922*a*, 1922*b*, 1927, 1934; Winge and Ditlevsen 1938, 1947; Haskins and Haskins 1951; Haskins et al. 1970
Oculatus	399			Schmidt 1920; Winge 1927
Armatus	1,412			Blacher 1927, 1928; Winge 1927; Haskins et al. 1970
Pauper	636			Winge 1927, 1934; Winge and Ditlevsen 1938, 1947; Haskins et al. 1970
Sanguineus	575			Winge 1927
Iridescens	256			Winge 1922*b*; Blacher 1928; Winge and Ditlevsen 1947; Dzwillo 1959
Aureus	105			Winge 1927
Variabilis	81			Winge 1927
Ferrugineus				Winge 1927
Bimaculatus	68			Blacher 1927, 1928
Reticulatus				Natali and Natali 1931 (in Kirpichnikov 1981)
Trimaculatus	78			Natali and Natali 1931 (in Kirpichnikov 1981)
Viridis				Natali and Natali 1931 (in Kirpichnikov 1981)
Bipunctatus				Natali and Natali 1931 (in Kirpichnikov 1981); Kirpichnikov 1935
Doppelschwert	609			Dzwillo 1959
Filigran	71			Dzwillo 1959
Orange area[b]				Houde 1992; Brooks and Endler 2001; Karino and Haijima 2001
Black area[b]				Brooks and Endler 2001
Fuzzy black area[b]				Brooks and Endler 2001
Iridescent area[b]				Brooks and Endler 2001
Mean brightness[b]				Brooks and Endler 2001
Brightness contrast[b]				Brooks and Endler 2001
Mean chroma[b]				Brooks and Endler 2001
Attractiveness[b]				Brooks 2000
Tail area[b]				Brooks and Endler 2001
Courtship[b]				Farr 1983
X linked:[a]				
Lineatus	76			Winge 1927, 1934
Nigrocaudatus I	21			Nybelin 1947
X and Y linked:				
Maculatus-black				Winge and Ditlevsen 1947; Haskins et al. 1961
Elongatus		4.31 (348)	6.43 (1,276)	Winge 1922*a*, 1927
Vitellinus		6.13 (1,321)	3.75 (800)	Winge 1927, 1934; Haskins et al. 1970
Coccineus		.33 (1,198)	.48 (414)	Winge 1927, 1934; Dzwillo 1959
Tigrinus		.21 (938)	2.91 (206)	Winge 1927, 1934
Luteus		.89 (1,012)	3.82 (157)	Winge 1927, 1934
Minutus			2.67 (487)	Winge 1927, 1934
Cinnamomeus				Winge 1927
Solaris			0 (20)	Kirpichnikov 1935
Caudomaculatus				Blacher 1928
Anterior rubra		20 (25)		Blacher 1928
Purpureus			0 (52)	Natali and Natali 1931 (in Kirpichnikov 1981); Kirpichnikov 1935
Lutescens				Natali and Natali 1931 (in Kirpichnikov 1981)
Nigrocaudatus II		0 (74)	4.00 (25)	Dzwillo 1959; Nayudu 1979
Flavus		0 (77)	0 (17)	Winge and Ditlevsen 1947; Nayudu 1979

Table 3 (*Continued*)

		Crossover frequency		
	N	X to Y % (*N*)	Y to X % (*N*)	Reference
Pigmentiert caudalis		5.19 (270)	7.42 (364)	Dzwillo 1959; Schröder 1969*a*; Nayudu 1979
Sb				Haskins et al. 1961
Red tail		2.06 (97)	1.64 (548)	Fernando and Phang 1990; Khoo et al. 1999*b*, 1999*c*
Blue tail		1.79 (280)		Fernando and Phang 1990; Phang and Fernando 1991
Green tail		0 (312)		Phang et al. 1989*a*; Phang and Fernando 1991; V. P. E. Phang, unpublished data
Snakeskin body			.36 (2,507)	Phang et al. 1989*a*, 1989*b*, 1990; Phang and Fernando 1991
Snakeskin tail			.11 (948)	Phang et al. 1989*a*, 1989*b*, 1990; Phang and Fernando 1991
Variegated tail		1.03 (679)	3.25 (462)	Khoo et al. 1999*a*, 1999*b*
Black caudal peduncle		2.73 (549)	2.56 (260)	Khoo et al. 1999*b*, 1999*c*
Autosomal:				
Zebrinus				Winge 1927
Bar				Phang et al. 1999
Blond				Goodrich et al. 1944
Golden				Goodrich et al. 1944
Blue				Dzwillo 1959
Albino				Haskins and Haskins 1948
Kalymma				Schröder 1969*b*
Suppressor				Schröder 1969*b*
Elongated				Horn 1972

Note: *N* is the number of offspring examined; in some cases, crossing over was detected only outside of controlled crosses.

[a] Not known to recombine.

[b] Denotes quantitative genetic analyses that indicate a Y-linked component.

have never been seen to recombine with the X, while others recombine at a rate of up to 8% (table 3). This rate of recombination is similar to the potential for recombination inferred from the analysis of synaptonemal complexes (2/49, or approximately 4%; Traut and Winking 2001). Crossover frequencies suggest that the recombining regions of the X and Y chromosomes are similar, since the same gene complex recombines from Y to X and from X to Y at a similar frequency (table 3; sign test, NS). Crossover rates have been interpreted in terms of a physical linkage map of the Y chromosome, with tight linkage of those nonrecombining Y-linked genes to a major male sex-determining locus or loci on the Y chromosome (Winge 1927). Recombination is suppressed in this region but increases with increasing distance from the sex-determining region. Suppression of recombination is probably not complete even in the nonhomologous region. Rare crossover events at a frequency of <1/3,800 (table 3) have occurred between the genes for the red and the black elements of the *Maculatus* color pattern, which are believed to be located very close to the sex-determining region (Winge 1934). A recent linkage map based on phenotypic traits suggested that the sex-determining region is flanked on both sides by recombining regions (Khoo et al. 1999*b*).

The X chromosome is less well understood but is assumed to carry similar genes to the Y, apart from those involved in sex determination of males, since YY males, which have no X chromosome, can be fully viable (Winge and Ditlevsen 1938; Haskins et al. 1970). The X chromosome may have a region homologous to that of the nonrecombining region of the Y, but so far no genes have been shown to be exclusively linked to it. Two genes for color patterns that are on the X but are not known to recombine to the Y (*Lineatus* and *Nigrocaudatus* I; table 3) are candidates for such a region, but the small sample sizes in these studies suggest instead that these genes would be found to recombine with the Y if more crosses were made, as has been the case for many other color genes. X-linked color patterns always have male-limited expression but can be developed in females with testosterone treatment, which allows confirmation of inheritance in females. Only patterns that have never been reported from wild populations show weak expression in females without testosterone treatment (*Nigrocaudatus* I and II, *Flavus*, *Pigmentiert caudalis*, red tail, blue tail, green tail, variegated tail, and black caudal peduncle; references in table 3) and are most likely mutations restricted to domesticated populations.

The autosomes have many fewer genes for pigmentation and fin morphology. *Zebrinus* and *Bar* are similar to the

sex-linked pigmentation traits in that expression is limited to males. The other known autosomal genes are expressed in both males and females (references in table 3).

These extensive studies using crossover rates and pedigree analysis of male coloration genes to map guppy sex chromosomes provide only an approximate picture of the linkage patterns of attractive characters in natural populations. First, estimates of sex linkage and rates of recombination in many cases were based on small sample sizes (table 3). More importantly, the extent to which color genes studied in domesticated strains represent genes found in natural populations is unknown.

The sex linkage of genes controlling attractive male characters has been directly measured in a few wild populations. By treating females with testosterone, Haskins et al. (1961) showed that some color patterns that were inherited on the X or the Y chromosome in low-predation populations were exclusively Y linked in high-predation populations. Thus, the low-predation populations that are characterized by higher levels of preference and elevated levels of male coloration are also those that exhibit color genes linked to the X chromosome. This result would be expected under our proposition that physical linkage between female preference genes and male coloration genes, facilitated by linkage of both types of characters to sex chromosomes, helps drive sexual selection in poeciliids.

Genetics of Female Preference in Guppies

We investigated the genetic inheritance of female preference by crossing and backcrossing populations with highly divergent levels of female preference and male attractive coloration. A low-coloration guppy population (PA6) was collected in 1997 from a coastal, high-predation stream (Rio San Miguel, a tributary of the Rio San Juan) 6 km outside of Caripito, Venezuela. The second parental strain was a population of a distinct and highly colorful form of guppy known to aquarists as Endler's live-bearer (ELB), collected from the coastal town of Cumana, Venezuela. This type has many orange and black patterns not observed in other guppy populations (H. Alexander and F. Breden, unpublished data). A recent survey of this region (H. Alexander and F. Breden, unpublished data) showed that ELB is endemic to only a few canals in Cumana.

Adult females from the two parental populations, female F1 offspring from reciprocal crosses (one with an ELB dam and one with a PA6 dam), and female offspring from backcrosses to the parental populations were tested for preference. Details of methods for measuring choice are given in the caption of figure 1. There was no indication of postmating reproductive incompatibility between these populations, and both F1 males and females produced offspring when backcrossed to the parental populations.

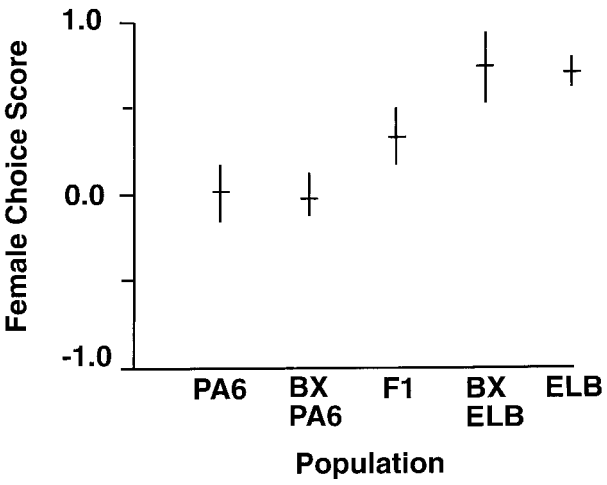

Figure 1: Mean and standard error of choice scores from low-coloration population (PA6), Endler's live-bearer population (ELB), F1 offspring from one set of reciprocal crosses, and backcrosses to parental populations. Choice tests were conducted in a 40-L aquarium partitioned by glass into a middle section, with bottom dimensions of 25 cm × 25 cm, and two end sections, with bottom dimensions of 12.5 cm × 25 cm. Males were placed in the end partitions, and the test female was placed in the center section. An 8-L dither tank containing female guppies was placed directly behind the center of the tank. The purpose of the dither fish was to calm the female during the acclimation and test periods and to allow her the option of schooling with conspecifics. The female and two males were allowed to equilibrate either for 3–5 h or overnight in the dark. A halogen light placed above the tank was then turned on automatically, and the fish were able to observe each other through the glass for an additional 1-h equilibration period. Following this equilibration period, an observer, facing the side of the aquarium behind a curtain into which a 5 × 15-cm hole had been cut, recorded whether the female was oriented toward a male whenever she was within 5 cm of his side of the tank. Behavior was recorded for 20 min. Females were tested either as virgins or within 48 h postparturition. The stimulus males were one PA6 and one ELB male chosen at random from a set of eight males from each population, matched to within 1 mm standard length; all ELB males were dramatically more colorful than PA6 males. Female preference for the ELB male was calculated using the following equation: $(X_{ELB} - X_{PA6})/(X_{ELB} + X_{PA6})$, where X_{ELB} and X_{PA6} are the total time a female was oriented toward the colorful ELB or less colorful PA6 male, respectively. This statistic ranges from −1.0 to 1.0, and positive values indicate a choice for the colorful ELB male.

Choice scores for females from the low-coloration PA6 population ranged from −0.83 to 0.86, and the average choice score was not significantly different from 0 ($\bar{X} = 0.05$, $N = 11$, $P > .2$, t-test; fig. 1). In contrast, all ELB females preferred the ELB male (choice scores ranged from 0.20 to 1.0), and overall there was a significant choice for the ELB male ($\bar{X} = 0.66$, $N = 11$, $P < .001$). These results support several studies that have shown a correlation between the level of male coloration and female preference in guppy populations (Breden and Stoner 1987; Stoner and Breden 1988; Houde and Endler 1990; Endler and

Houde 1995). The female choice scores for the F1 females showed an intermediate value ($\bar{X} = 0.36$, $N = 13$), and most importantly, the backcross scores showed regression toward the parental population values ($\bar{X} = -0.07$, $N = 7$, backcross to PA6; $\bar{X} = 0.69$, $N = 3$, backcross to ELB). Because only one set of reciprocal crosses has been tested, and because the sample sizes for each type of F1 and backcross populations are small, it is premature to quantify the results of these crosses in terms of additive and maternal effects. However, these results do show that there is a strong additive genetic component to the difference between these populations and that it will be possible to study the inheritance of guppy female preference in the types of crosses and backcrosses necessary for quantitative trait locus studies. We are currently using microsatellite markers for the guppy anchored on the dense *Xiphophorus* linkage map (S. Kazianis, F. Breden, and R. B. Walter, unpublished data) to test for a large X chromosome effect on female preference in these populations.

In conclusion, organisms with evolving sex chromosomes can show variation in the number of functional genes linked to the sex chromosomes, variation in recombination rates along sex chromosomes, and variation in sex-determination system, such as male or female heterogamety or number of alleles at the sex-determining locus. Such systems provide an ideal opportunity to study the effect of sex linkage on the evolution of behavior and the effect of differences in behavioral strategies between males and females on genomic organization. For example, the forces driving the evolution of sexually antagonistic genes may select for the retention of regions of sex chromosomes with functional genes. Ultimately, modern molecular genetic techniques and genomic data in conjunction with well-studied model behavioral systems will enable us to examine how behavioral evolution is constrained by genetic processes and, in turn, how behavioral processes can constrain the evolution of genomic structure.

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Literature Cited

Alvesalo, L. 1997. Sex chromosomes and human growth: a dental approach. Human Genetics 101:1–5.

Angus, R. A. 1989. A genetic overview of poeciliid fishes. Pages 51–68 *in* G. K. Meffe and F. F. Snelson, Jr., eds. Ecology and evolution of livebearing fishes (Poeciliidae). Prentice Hall, Englewood Cliffs, N.J.

Barton, N. H., and M. Turelli. 1991. Natural and sexual selection on many loci. Genetics 127:229–255.

Bischoff, R. J., J. L. Gould, and D. I. Rubenstein. 1985. Tail size and female choice in the guppy (*Poecilia reticulata*). Behavioral Ecology and Sociobiology 17:253–255.

Blacher, L. J. 1927. Materials for the genetics of *Lebistes reticulatus* Peters. Transactions of the Laboratory of Experimental Biology of the Zoopark of Moscow 3: 139–152.

———. 1928. Materials for the genetics of *Lebistes reticulatus*. II. Transactions of the Laboratory of Experimental Biology of the Zoopark of Moscow 4:245–253.

Black, D. A., and W. M. Howell. 1979. The North American mosquitofish, *Gambusia affinis*: a unique case in sex chromosome evolution. Copeia 1979:509–513.

Borowsky, R. 1984. The evolutionary genetics of *Xiphophorus*. Pages 235–310 *in* B. J. Turner, ed. Evolutionary genetics of fishes. Plenum, New York.

———. 1987. Genetic polymorphism in adult male size in *Xiphophorus variatus* (Atheriniformes: Poeciliidae). Copeia 1987:782–787.

Breden, F., and G. Stoner. 1987. Male predation risk determines female preference in the Trinidad guppy. Nature 329:831–833.

Brooks, R. 2000. Negative genetic correlation between male sexual attractiveness and survival. Nature 406: 67–70.

Brooks, R., and J. A. Endler. 2001. Direct and indirect sexual selection and quantitative genetics of male traits in guppies (*Poecilia reticulata*). Evolution 55:1002–1015.

Bull, J. J. 1983. Evolution of sex determining mechanisms. Benjamin Cummings, Menlo Park, Calif.

Charlesworth, B. 1978. A model for the evolution of Y chromosomes and dosage compensation. Proceedings of the National Academy of Sciences of the USA 75: 5618–5622.

Charlesworth, B., J. A. Coyne, and N. H. Barton. 1987. The relative rates of evolution of sex chromosomes and autosomes. American Naturalist 130:113–146.

de Zulueta, A. 1925. La herencia ligada al sexo en el coleóptero *Phytodecta variabilis* (Ol.). Eos 1:203–229.

Dzwillo, M. 1959. Genetische Untersuchungen an domestizierten Stämmen von *Lebistes reticulatus* (Peters). Mitteilungen aus dem Hamburgischen Zoologischen Museum und Institut 57:143–186.

Endler, J. A. 1980. Natural selection on color patterns in *Poecilia reticulata*. Evolution 34:76–91.

Endler, J. A., and A. E. Houde. 1995. Geographic variation

in female preferences for male traits in *Poecilia reticulata*. Evolution 49:456–468.

Erbelding-Denk, C., J. H. Schröder, M. Schartl, I. Nanda, M. Schmid, and J. T. Epplen. 1994. Male polymorphism in *Limia perugiae* (Pisces: Poeciliidae). Behavior Genetics 24:95–101.

Farr, J. A. 1980. Social behavior patterns as determinants of reproductive success in the guppy, *Poecilia reticulata* Peters (Pisces: Poeciliidae). Behaviour 74:38–91.

———. 1983. The inheritance of quantitative fitness traits in guppies, *Poecilia reticulata* (Pisces: Poeciliidae). Evolution 37:1193–1209.

Fernando, A. A., and V. P. E. Phang. 1990. Inheritance of red and blue caudal fin colourations in two domesticated varieties of the guppy, *Poecilia reticulata*. Journal of Aquaculture in the Tropics 5:209–217.

Fisher, R. A. 1931. The evolution of dominance. Biological Reviews 6:345–368.

———. 1958. The genetical theory of natural selection. Dover, New York.

Fujio, Y., M. Nakajima, and Y. Nagahama. 1990. Detection of a low temperature-resistant gene in guppy (*Poecilia reticulata*), with reference to sex-linked inheritance. Japanese Journal of Genetics 65:201–207.

Galán, F. 1931. Estudios sobre la espermatogénesis del coleóptero *Phytodecta variabilis* (Ol.). Eos 7:461–501.

Goodrich, H. B., N. D. Josephson, J. P. Trinkhaus, and J. M. Slate. 1944. The cellular expression and genetics of two new genes in *Lebistes reticulatus*. Genetics 29: 584–592.

Gosler, A. G., P. R. Barnett, and S. J. Reynolds. 2000. Inheritance and variation in eggshell patterning in the great tit *Parus major*. Proceedings of the Royal Society of London B, Biological Sciences 267:2469–2473.

Graves, J. A. M. 1995. The origin and function of the mammalian Y chromosome and Y-borne genes: an evolving understanding. BioEssays 17:311–321.

Haskins, C. P., and E. F. Haskins. 1948. Albinism, a semilethal autosomal mutation in *Lebistes reticulatus*. Heredity 2:251–262.

———. 1951. The inheritance of certain color patterns in wild populations of *Lebistes reticulatus* in Trinidad. Evolution 5:216–225.

Haskins, C. P., E. F. Haskins, J. J. A. McLaughlin, and R. E. Hewitt. 1961. Polymorphism and population structure in *Lebistes reticulatus*, an ecological study. Pages 320–395 *in* W. F. Blair, ed. Vertebrate speciation. University of Texas Press, Austin.

Haskins, C. P., P. Young, R. E. Hewitt, and E. F. Haskins. 1970. Stabilised heterozygosis of supergenes mediating certain Y-linked colour patterns in populations of *Lebistes reticulatus*. Heredity 25:575–589.

Horn, P. 1972. A mindkét ivarú guppin (*Poecilia reticulata Peters*, 1859) mutatkozó új autoszomális domináns mutáció. Allattani Kozlemenyek 59:53–59.

Hornaday, K., S. Alexander, and F. Breden. 1994. Absence of repetitive DNA sequences associated with sex chromosomes in natural populations of the Trinidad guppy (*Poecilia reticulata*). Journal of Molecular Evolution 39: 431–433.

Houde, A. E. 1992. Sex-linked heritability of a sexually selected character in a natural population of *Poecilia reticulata* (Pisces: Poeciliidae) (guppies). Heredity 69: 229–235.

Houde, A. E., and J. A. Endler. 1990. Correlated evolution of female mating preferences and male color patterns in two populations of guppies (*Poecilia reticulata*). Science (Washington, D.C.) 248:1405–1408.

Kallman, K. D. 1970. Sex determination and the restriction of sex-linked pigment patterns to the X and Y chromosomes in populations of a poeciliid fish, *Xiphophorus maculatus*, from the Belize and Sibun Rivers of British Honduras. Zoologica (New York) 55:1–16.

———. 1975. The platyfish, *Xiphophorus maculatus*. Pages 81–132 *in* R. C. King, ed. Handbook of genetics. Vol. 4. Plenum, New York.

———. 1983. The sex determining mechanism of the poeciliid fish, *Xiphophorus montezumae*, and the genetic control of the sexual maturation process and adult size. Copeia 1983:755–769.

———. 1989. Genetic control of size at maturity in *Xiphophorus*. Pages 163–184 *in* G. K. Meffe and F. F. Snelson, Jr., eds. Ecology and evolution of livebearing fishes (Poeciliidae). Prentice Hall, Englewood Cliffs, N.J.

Kallman, K. D., and V. Borkoski. 1978. A sex-linked gene controlling the onset of sexual maturity in female and male platyfish (*Xiphophorus maculatus*), fecundity in females and adult size in males. Genetics 89:79–119.

Kallman, K. D., and R. Borowsky. 1972. The genetics of gonopodial polymorphism in two species of poeciliid fish. Heredity 28:297–310.

Karino, K., and Y. Haijima. 2001. Heritability of male secondary sexual traits in feral guppies in Japan. Journal of Ethology 19:33–37.

Kazianis, S., H. Gutbrod, R. S. Nairn, B. B. McEntire, L. D. Coletta, R. B. Walter, R. L. Borowsky, et al. 1998. Localization of a *CDKN2* gene in linkage group V of *Xiphophorus* fishes defines it as a candidate for the *DIFF* tumor suppressor. Genes Chromosomes & Cancer 22: 210–220.

Khoo, G., T. M. Lim, W.-K. Chan, and V. P. E. Phang. 1999*a*. Genetic basis of the variegated tail pattern in the guppy, *Poecilia reticulata*. Zoological Science (Tokyo) 16: 431–437.

———. 1999*b*. Linkage analysis and mapping of three sex-

linked color pattern genes in the guppy, *Poecilia reticulata*. Zoological Science (Tokyo) 16:893–903.

———. 1999*c*. Sex-linkage of the black caudal-peduncle and red tail genes in the tuxedo strain of the guppy, *Poecilia reticulata*. Zoological Science (Tokyo) 16: 629–638.

Kirkpatrick, M., and N. H. Barton. 1997. Strength of indirect selection on female mating preference. Proceedings of the National Academy of Sciences of the USA 94:1282–1286.

Kirpichnikov, V. S. 1981. Genetic bases of fish selection. Translated by G. G. Gause. Springer, Berlin.

Kirpichnikov, W. 1935. Autosomal genes in *Lebistes reticulatus* and the problem of the arising of the genetic sex determination. Biologicheskii Zhurnal (Moscow) 4: 343–354.

Kirsch, S., B. Weiss, M. De Rosa, T. Ogata, G. Lombardi, and G. A. Rappold. 2000. FISH deletion mapping defines a single location for the Y chromosome stature gene, *GCY*. Journal of Medical Genetics 37:593–599.

Lahn, B. T., and D. C. Page. 1997. Functional coherence of the human Y chromosome. Science (Washington, D.C.) 278:675–679.

Lande, R. 1981. Models of speciation by sexual selection on polygenic traits. Proceedings of the National Academy of Sciences of the USA 78:3721–3725.

Lande, R., and G. S. Wilkinson. 1999. Models of sex-ratio meiotic drive and sexual selection in stalk-eyed flies. Genetical Research 74:245–253.

Matsuda, M., C. Matsuda, S. Hamaguchi, and M. Sakaizumi. 1998. Identification of the sex chromosomes of the medaka, *Oryzias latipes*, by fluorescence in situ hybridization. Cytogenetics and Cell Genetics 82:257–262.

Nanda, I., W. Feichtinger, M. Schmid, J. H. Schröder, H. Zischler, and J. T. Epplen. 1990. Simple repetitive sequences are associated with differentiation of the sex chromosomes in the guppy fish. Journal of Molecular Evolution 30:456–462.

Nayudu, P. L. 1979. Genetic studies of melanic color patterns and atypical sex determination in the guppy, *Poecilia reticulata*. Copeia 1979:225–231.

Nichols, R. A., and R. K. Butlin. 1989. Does runaway sexual selection work in finite populations? Journal of Evolutionary Biology 2:299–313.

Nicoletto, P. F. 1993. Female sexual response to condition-dependent ornaments in the guppy, *Poecilia reticulata*. Animal Behaviour 46:441–450.

Nielsen, J. K. 1997. Genetics of the ability of *Phyllotreta nemorum* larvae to survive in an atypical host plant, *Barbarea vulgaris* ssp. *arcuata*. Entomologia Experimentalis et Applicata 82:37–44.

Nybelin, O. 1947. Ett fall av X-bunden nedärvning hos *Lebistes reticulatus* (Peters). Zoologiska Bidrag fran Uppsala 25:448–454.

Ohno, S. 1974. Animal cytogenetics. Vol. 4. Chordata 1. Borntraeger, Berlin.

Phang, V. P. E., and A. A. Fernando. 1991. Linkage analysis of the X-linked green tail and blue tail color genes in the guppy. Zoological Science (Tokyo) 8:975–981.

Phang, V. P. E., L. N. Ng, and A. A. Fernando. 1989*a*. Genetics of the colour of the yellow snakeskin variety of the guppy, *Poecilia reticulata*. Singapore Journal of Primary Industries 17:19–28.

———. 1989*b*. Inheritance of the snakeskin color pattern in the guppy, *Poecilia reticulata*. Journal of Heredity 80: 393–399.

Phang, V. P. E., A. A. Fernando, and E. W. K. Chia. 1990. Inheritance of the color patterns of the blue snakeskin and red snakeskin varieties of the guppy, *Poecilia reticulata*. Zoological Science (Tokyo) 7:419–425.

Phang, V. P. E., G. Khoo, and S. P. Ang. 1999. Interaction between the autosomal recessive *bar* gene and the Y-linked snakeskin body (*Ssb*) pattern gene in the guppy, *Poecilia reticulata*. Zoological Science (Tokyo) 16: 905–908.

Rauchenberger, M., K. D. Kallman, and D. C. Morizot. 1990. Monophyly and geography of the Río Pánuco Basin swordtails (genus *Xiphophorus*) with descriptions of four new species. American Museum Novitates 2975: 1–41.

Reynolds, J. D., and M. R. Gross. 1992. Female mate preference enhances offspring growth and reproduction in a fish, *Poecilia reticulata*. Proceedings of the Royal Society of London B, Biological Sciences 250:57–62.

Rice, W. R. 1984. Sex chromosomes and the evolution of sexual dimorphism. Evolution 38:735–742.

———. 1987*a*. The accumulation of sexually antagonistic genes as a selective agent promoting the evolution of reduced recombination between primitive sex chromosomes. Evolution 41:911–914.

———. 1987*b*. Genetic hitchhiking and the evolution of reduced genetic activity of the Y sex chromosome. Genetics 116:161–167.

Schmidt, J. 1920. Racial investigations. IV. The genetic behaviour of a secondary sexual character. Comptes Rendus des Travaux de Laboratoire Carlsberg 14(8): 1–12.

Schröder, J. H. 1969*a*. Inheritance of fin characters in the guppy (*Lebistes reticulatus* Peters). Theoretical and Applied Genetics 39:73–78.

———. 1969*b*. Radiation-induced spermatogonial exchange between the X and Y chromosomes in the guppy. Canadian Journal of Genetics and Cytology 11:948–951.

Scriber, J. M., R. H. Hagen, and R. C. Lederhouse. 1996. Genetics of mimicry in the tiger swallowtail butterflies,

Papilio glaucus and *P. canadensis* (Lepidoptera: Papilionidae). Evolution 50:222–236.

Seehausen, O., J. J. M. van Alphen, and R. Lande. 1999. Color polymorphism and sex ratio distortion in a cichlid fish as an incipient stage in sympatric speciation by sexual selection. Ecology Letters 2:367–378.

Segarra, C., and E. Petitpierre. 1990. Chromosomal survey in three genera of Alticinae (Coleoptera, Chrysomelidae). Cytobios 64:169–174.

Selmanoff, M. K., J. E. Jumonville, S. C. Maxson, and B. E. Ginsburg. 1975. Evidence for a Y chromosomal contribution to an aggressive phenotype in inbred mice. Nature 253:529–530.

Sluyter, F., G. A. van Oortmerssen, and J. M. Koolhaas. 1994. Studies on wild house mice. VI. Differential effects of the Y chromosome on intermale aggression. Aggressive Behaviour 20:379–386.

Smith, S. G., and N. Virkki. 1978. Animal cytogenetics. Vol. 3. Insecta 5. Borntraeger, Berlin.

Stoner, G., and F. Breden. 1988. Phenotypic variation in female choice related to geographic variation in male predation risk in the Trinidad guppy, *Poecilia reticulata*. Behavioral Ecology and Sociobiology 22:285–291.

Traut, W., and H. Winking. 2001. Meiotic chromosomes and stages of sex chromosome evolution in fish: zebrafish, platyfish and guppy. Chromosome Research 9: 659–72.

Travis, J. 1994. Size-dependent behavioral variation and its genetic control within and among populations. Pages 165–187 *in* C. Boake, ed. Quantitative genetic studies of behavioural evolution. University of Chicago Press, Chicago.

Wada, H., A. Shimada, S. Fukamachi, K. Naruse, and A. Shima. 1998. Sex-linked inheritance of the *If* locus in the medaka fish (*Oryzias latipes*). Zoological Science (Tokyo) 15:123–126.

Winge, Ö. 1922*a*. Crossing-over between the X- and the Y-chromosome in *Lebistes*. Comptes Rendus des Travaux du Laboratoire Carlsberg 14(20):1–20.

———. 1922*b*. One-sided masculine and sex-linked inheritance in *Lebistes reticulatus*. Journal of Genetics 12: 146–162.

———. 1927. The location of eighteen genes in *Lebistes reticulatus*. Journal of Genetics 18:1–43.

———. 1934. The experimental alteration of sex chromosomes into autosomes and vice versa, as illustrated by *Lebistes*. Comptes Rendus des Travaux du Laboratoire Carlsberg 21:1–49.

Winge, Ö., and E. Ditlevsen. 1938. A lethal gene in the Y chromosome of *Lebistes*. Comptes Rendus des Travaux du Laboratoire Carlsberg 22:203–210.

———. 1947. Colour inheritance and sex determination in *Lebistes*. Heredity 1:65–83.

Yamanaka, M., M. Nakajima, and Y. Fujio. 1995. Estimation of the number of loci controlling the male body size in the guppy. Tohoku Journal of Agricultural Research 46:21–28.

Zander, C. D. 1968. Über die Vererbung von Y-gebunden Farbgenen des *Xiphophorus pygmaeus nigrensis* Rosen (Pisces). Molecular & General Genetics 101:29–42.

Zimmerer, E. J., and K. D. Kallman. 1988. The inheritance of vertical barring (aggression and appeasement signals) in the pygmy swordtail, *Xiphophorus nigrensis* (Poeciliidae, Teleostei). Copeia 1988:299–307.

———. 1989. Genetic basis for alternative reproductive tactics in the pygmy swordtail, *Xiphophorus nigrensis*. Evolution 43:1293–1307.

Chapter 3

Relative fitness of alternative male reproductive tactics in a mammal varies between years

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Relative fitness of alternative male reproductive tactics in a mammal varies between years

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Summary

1. In many species, males can use different behavioural tactics to achieve fertilization, so-called alternative reproductive tactics (ARTs). Few field studies have measured fitness consequences of ARTs under varying environmental conditions.

2. Here, we describe fitness consequences of three phenotypically plastic ARTs in the African striped mouse (*Rhabdomys pumilio*) and show that relative fitness of ARTs differs between years. Each year represents a different generation.

3. For the generation living under high population density, tactics differed in relative fitness in accordance with the theory of conditional strategies, with highly successful territorial breeding males having 10 times higher success than solitary roaming males and 102 times higher success than adult natally philopatric males.

4. For the generation living under intermediate population density, the territorial breeding and roaming tactics yielded similar fitness, which would be in agreement with the theory of mixed strategies. No philopatric males occurred.

5. For the generation living under low population density, roaming was the only tactic used and some roamers had very high fitness.

6. The main prediction of status-dependent selection for conditional strategies is a correlation between fitness and status, often measured as body mass, but we did not find this correlation within tactics when more than one tactic was expressed in the population.

7. Female distribution seems to have an effect on which reproductive tactics male chose: female defence polygyny when females are clumped (interference competition), but a searching tactic when females are dispersed (scramble competition). In contrast to predictions arising from theory on scramble competition, male body mass was important in determining fitness only in the year when females were dispersed, but not in other years.

8. Our results indicate that the differentiation between conditional and mixed strategies is not an absolute one. In many other species, environmental conditions might fluctuate temporally and spatially so that the normally suboptimal tactic yields similar fitness to the (usually) dominant tactic or that only a single tactic prevails.

9. We suggest the term single strategy, independent of current fitness consequences, for systems where tactics are not genetically determined, in contrast to genetically determined alternative strategies.

Key-words: female choice, prairie vole, scramble competition, social flexibility

Introduction

Reproductive tactics of males are influenced by the spatial distribution of females, with a clumped distribution favouring harem defence polygyny (interference competition), and a dispersed distribution favouring a male searching tactic (scramble competition; Emlen & Oring 1977; Lane *et al.* 2009; Shuster & Wade 2003). However, individual males can differ in their reproductive tactics to maximize fitness. Such alternative reproductive tactics (ARTs) have been described in vertebrates and invertebrates, and they are more common in males than in females (Taborsky, Oliveira & Brockmann

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2008). While ARTs have been reported for more than 100 species, fitness consequences have been measured in only a few species, most often with genetically determined ARTs (e.g. Lank *et al*. 1995; Shuster & Sassaman 1997). We still know relatively little about how ARTs do or do not differ in fitness, which is critical to understanding how they evolved and how they are maintained (Taborsky, Oliveira & Brockmann 2008).

Alternative reproductive tactics are the result of underlying strategies. A strategy describes the decision rules of an individual, whereas a tactic is the behaviour resulting from these decision rules (Krebs & Davies 1993). Gross (1996) defined three different strategies. In alternative strategies, ARTs are genetically polymorphic and different tactics yield the same average fitness. Such genetically determined ARTs have been documented in fish (Taborsky 2008a), lizards (Moore, Hews & Knapp 1998), ruffs (*Philomachus pugnax*; Lank *et al*. 1995) and the isopod *Paracerceis sculpta* (Shuster & Sassaman 1997). In contrast, in mixed strategies, individuals are able to express all tactics. Crucially, alternative and mixed strategies are both characterized by frequency-dependent selection resulting in equal fitness between tactics. However, to our knowledge, no convincing example for a mixed strategy has been found (Gross 1996). In most cases, different tactics are believed to differ in fitness and are based on conditional strategies (Gross 1996). In species with conditional strategies, the tactic that an individual chooses depends on its competitive abilities, leading to status-dependent selection. The most competitive individuals follow the tactic that yields the greatest fitness pay-off, called the bourgeois tactic. Less-competitive males, which are often smaller and younger than the bourgeois males, make the best of a bad job (Dawkins 1980), following a tactic (often called sneaker or satellite) with low fitness that is still better than no reproductive success at all. These males change to the bourgeois tactic when they grow larger. To understand the evolution of ARTs, it is crucial to understand the fitness pay-offs of the different tactics to predict when and why individuals change their tactic: when do fitness functions of different tactics cross, such that a switch-point from one tactic to another is reached (Taborsky 2008b)?

Males of the African striped mouse (*Rhabdomys pumilio*) follow one of the three ARTs: group living territorial breeder, group living natally philopatric male or solitarily living roaming male. Males can switch tactics during their lives (Schradin *et al*. 2009). Relative body mass appears to determine tactic as philopatric males are small, roaming males are intermediate in mass and territorial breeders are the heaviest. Upon gaining mass, philopatrics can change into roamers or territorial breeders, and roamers can become territorial breeders (Schradin *et al*. 2009). Males also follow alternative dispersal tactics, with larger males dispersing over shorter distances, smaller males often being forced to disperse large distances over suboptimal habitat (Solmsen, Johannesen & Schradin 2011). Males typically remain natally philopatric during the breeding season they are born and have the chance to reproduce as roamers or territorial males during the next breeding season. They gain weight the season they are born and the winter preceding the next breeding season, when it rains and plant growth occurs. Large philopatrics might immigrate into groups that lost their breeding male (e.g. because of predation) any time of the year. Males that remained philopatric until the start of the next breeding season then might either remain philopatric or become roamers, and during the breeding season, males can switch tactic as their body mass increases (Schradin *et al*. 2009). However, adults experience only one breeding season; therefore, they cannot delay reproduction to another year when they might be heavier. This also means that every year of field study investigates a new generation.

We predict that fitness in the striped mouse is status (condition) dependent, with breeders having the highest fitness, roamers intermediate fitness and philopatrics the lowest fitness. We also predict that within tactics, fitness depends on body mass (status-dependent selection) because heavier males are more likely to win male–male competitions (Schradin 2004) and male body mass is known to be important in other polygynous mammals (Heske & Ostfeld 1990; Adrian *et al*. 2008). We expect that territorial breeder and roamer-specific fitness functions intersect, at a switch-point located between the mean body mass for territorial breeders and roamers (Fig. 1a).

One of the most interesting features of ARTs of the African striped mouse is that they vary with environmental conditions. The three tactics co-occur when population density is high. However, in a year with very low population density when females were dispersed as single breeders, all males followed the roaming tactic (Schradin, König & Pillay 2010). Under such environmental conditions, one would expect scramble competition and selection favouring traits like search ability and not body mass. Additionally, as single breeding females are a valuable resource, large roamers might be better able to monopolize them during the day of oestrus such that body mass and fitness would be positively correlated (Fig. 1c).

At intermediate population density, both solitary and communally breeding females occur as well as territorial breeding and roaming males but no adult philopatric males are present in the population. Importantly, under these conditions, roaming and territorial males do not differ in body mass. If body mass is a good proxy of competitive ability, and males were thus not differing in status, we predict that roaming and territorial males do not differ in reproductive success under these specific environmental conditions. We still expect fitness to be dependent on body mass, as heavier males should be more successful in male–male competition (Fig. 1b).

The tactic that male striped mice follow depends on population density (Schradin, König & Pillay 2010). In the current study, we choose 3 years differing strongly in population density to test theoretical assumptions of the fitness consequences for different tactics. We therefore test whether the fitness functions of male African striped mice correspond to predictions arising from status-dependent selection.

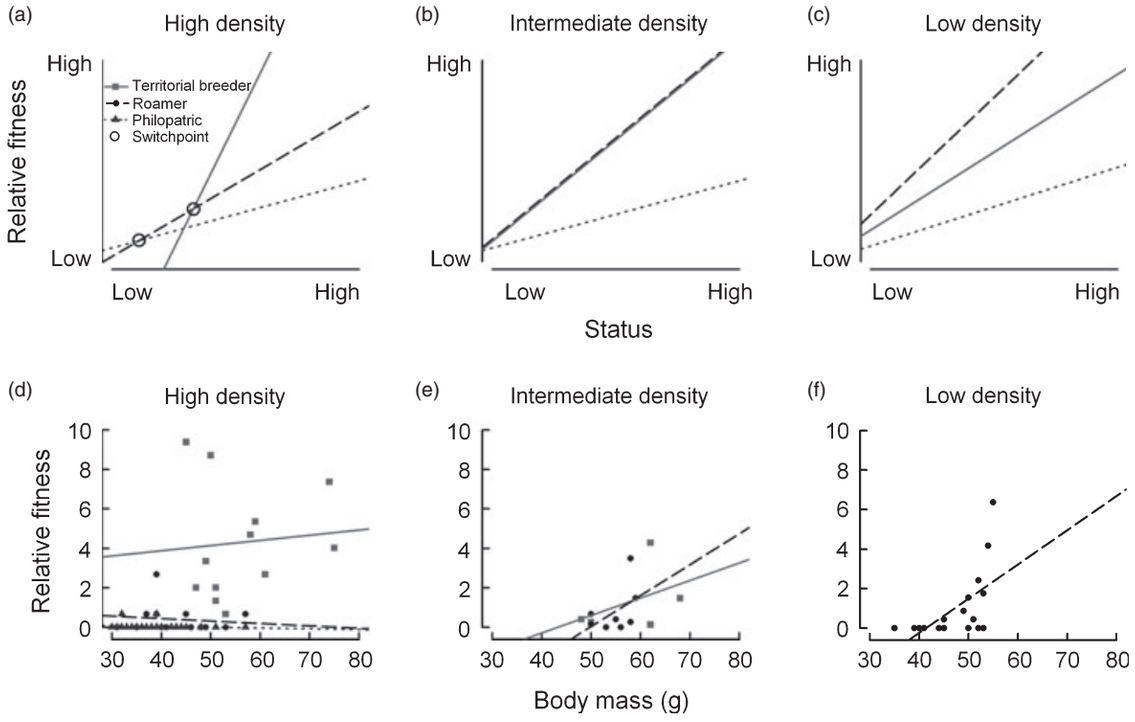

Fig. 1. (a–c) Predictions arising from status-dependent selection (derived from Fig. 1.3. in Taborsky, Oliveira & Brockmann 2008). For each year, we predicted fitness to be dependent on body mass (status), i.e. that heavier males were more successful. (a) Under high population density, heavy males were predicted to follow the tactic of territorial breeder, giving the highest fitness, intermediate mass males to be solitary roamers with intermediate fitness and small males to be philopatrics with low fitness. Predicted switch-points are marked. (b) Under intermediate population density when territorial breeders and roamers did not differ in body mass, we predicted the fitness functions for roamers and territorial breeders to be identical, and the fitness line of philopatrics is always below the other lines. No switch-points occur. (c) Under low population density, the fitness line for roamers is always above the lines for territorial breeders and philopatrics. No switch-points occur. (d–f) Results. Least-square linear fits of mass vs. relative fitness for tactics occurring each year. (d) Territorial breeders had higher fitness than roamers and philopatrics had the lowest fitness, but fitness did not increase with mass. The slopes of these regressions are not significantly different from zero (territorial breeding males: $t_{1,10} = 0{\cdot}28$, $P > 0{\cdot}78$; roamers: $t_{1,13} = 0{\cdot}45$, $P > 0{\cdot}66$; philopatrics: $t_{1,30} = 0{\cdot}80$, $P > 0{\cdot}42$). (e) No philopatrics were present. Territorial breeders had similar fitness to roamers, but fitness did not increase with body mass. Neither regression was significant (territorial breeding males: $t_{1,3} = 0{\cdot}84$, $P > 0{\cdot}46$; roamers: $t_{1,6} = 1{\cdot}31$, $P > 0{\cdot}23$). (f) Only one tactic occurred. Body mass predicted fitness ($t_{1,16} = 2{\cdot}96$, $P < 0{\cdot}01$).

Materials and methods

STUDY AREA AND PERIOD

The study was conducted in the Goegap Nature Reserve in South Africa (S 29 41.56, E 18 1.60). The vegetation type is Succulent Karoo, an arid area, with an average rainfall of 160 mm p.a. Most rain occurs in autumn/winter from April until July, some rain falls in spring (August/September), while summer (December–March) is the dry season.

Data were collected during 1 year with very low population density (2003: 1·5 mice ha^{-1}), 1 year with intermediate population density (2007: 6·5 mice ha^{-1}) and 1 year with high population density (2005: 19·0 mice ha^{-1}). The low population density in 2003 was because of an extreme winter drought and is the year with the lowest population density in our long-term study, but represents the condition found in other habitats with low population density (Schradin & Pillay 2005b). In 2003, all females were breeding solitarily, and in 2005 all females were breeding in communal groups, while in 2007 both communal groups and solitary breeding females occurred (Table 1). The size of the study area changed from 16·0 ha in 2003 to 8·8 ha in 2005 to 7·4 ha in 2007.

STUDY SPECIES

Striped mice are diurnal, inhabit an open habitat and are readily habituated to the presence of observers, which allows direct behavioural observations in the field (Schradin 2006). The breeding season of 3–4 months occurs in spring and depends on rainfall (Schradin & Pillay 2005a). In the current study, breeding took place from September to December in 2003, and from August to November during the years 2005 and 2007.

DETERMINATION OF MALE TACTICS

In our study, we considered all males with a body mass above 30 g and that were older than 4 weeks as reproductively mature (Brooks 1982). Male tactics were determined by a combination of trapping, behavioural observations and radiotracking (for sample sizes, see Table 1). Trapping was done around nesting sites at least 3 days per month. Trapped mice were weighed and sexed; males were recorded as scrotal (testes descended) or not (testes inside the body), and all mice were permanently marked with ear tags (National Band and Tag Co., Ontario, USA) and hair dye (Rapido, Pinetown, South Africa) for individual recognition during behavioural observations.

Table 1. Demographic descriptors of the study population and sample sizes for paternity studies during the three study years

Population density (striped mice per ha)	Year	Number of single females	Number of groups of communally breeding females	Sex ratio (males : females)	Ratio adult males : communally breeding female groups	Offspring	Litters > 1 pup	Philopatric males present at start of breeding season	Philopatric males born during breeding season	Roamers	Territorial breeders	Neighbouring males (tactic unknown)
19·0	2005	0	10	2·7	5·6	125	24	32 (55%)	41	15 (25%)	12 (20%)	10
6·5	2007	10	7	0·7	1·9	105	24	0 (0%)	0	8 (62%)	5 (38%)	0
1·5	2003	9	0	1·1	0·0	89	28	0 (0%)	46	18 (100%)	0 (0%)	0

Each nest was observed for 2–3 days in a row both during mornings and afternoons for 45 min at least every 2 weeks to determine group composition.

All territorial breeders and roamers were equipped with MD-2C radiotransmitters (Holohil, Canada) and radiotracked as described elsewhere (Schradin & Pillay 2005b) to determine home ranges (data presented in Schradin *et al.* 2009) and sleeping sites. Not all philopatrics (which only occurred in 2005) were radiocollared, as their tactic could be reliably determined by behavioural observations at their natal nests.

We classified males as philopatric if they had been trapped as juveniles (< 30 g) at a specific group and were as adults observed at the same group. Roaming males were classified as adult males that did not permanently share nesting sites with any other mice, although they might have spent some nights with single breeding females. Territorial breeding males were adult males that lived in groups other than their natal group. The latter were always the heaviest males of the groups, and each communal group had only one territorial breeding male. Males could only show one tactic at a time, i.e. they were either radiotracked > 50% of the time sleeping alone (roamers), or > 60% of the time sleeping with one group that was not their natal group (territorial breeders), or they were only trapped and observed at their natal group (philopatrics).

PATERNITY ANALYSIS

Through extensive trapping, we were able to obtain tissue samples (tail clips) for genetic analyses from all juveniles observed at the field site and from every observed adult male and female (Table 1). We isolated DNA from mouse tissue using magnetic particle purification (BioSprint 96 DNA Blood kit; Qiagen, Newport, KY, USA). We used nine polymorphic microsatellite loci from the house mouse genome (Teschke *et al.* 2008) and amplified them using two multiplexes. Both the first (Chr13_1, Chr1_12, Chr1_21, Chr2_3, Chr7_64) and the second multiplex (D3Mit211, Chr11_81, Chr19_18, Chr5_38) were amplified using the Qiagen PCR Multiplex kit with a final concentration of 0·1/0·2 μM primer for 35 cycles at an annealing temperature of 60 °C. Mean number of alleles per locus was 16·8 ± 4·3 (SD). In each year, one locus was identified in Cervus as being out of Hardy–Weinberg equilibrium, but the identity of the locus was different each time, so we retained all loci for parentage analysis. Typing error rates were estimated by amplifying and genotyping 153 individuals at the nine loci twice and calculated as the number of alleles that were scored differently between the two PCR amplifications divided by the total number of scored alleles. The observed error rate of 0·014 was strongly influenced by poor repeatability of amplification of one locus in one 96-well plate; if this one plate was excluded from the average, the average error rate then fell below 0·01.

Parentage analyses were performed separately for each year using Cervus 3.0 (Kalinowski, Taper & Marschall 2007). Parameters for the simulation of parentage analysis were set as 100 000 offspring, 95% sampling of candidate mothers, 85% sampling of candidate fathers (to be conservative), 0·015 proportion of loci mistyped (to be conservative) and the confidence level was set at a conservative 95%. Proportion of loci typed was 0·988 (2003), 0·975 (2005) and 0·978 (2007). We accepted parentage assignment when trio confidence was 95% and there was zero or one mismatch between each parent and offspring, and no more than two mismatches in the trio of candidate parents and offspring. If trio confidence was less than 95% but a parent–offspring pair met the 95% confidence threshold with one or fewer mismatches, we accepted the maternity

or paternity. If both a mother and father of the same offspring could be separately assigned with 95% confidence and one or fewer pair mismatches, but the trio had a confidence value of less than 95% and/or had more than two trio mismatches, we awarded parentage to the putative father if its pair delta value with the offspring exceeded that of the putative mother, and *vice versa*. Success in maternity assignment was 82·0% in 2003, 84·0% in 2005 and 97·1% in 2007. Success in paternity assignment was 92·4% in 2003, 78·0% in 2005 and 93·3% in 2007.

INFLUENCE OF THE SIRE'S TACTIC ON HIS SON'S TACTIC?

In total, we had 155 sons, of which 24 were still present as adults at our field site the next breeding season. We determined the reproductive tactic of these males as adults. Sample sizes were too low to directly estimate heritability (h^2). To test whether roamers were more likely to sire roamers and territorial breeders more likely to sire territorial breeders, we compared the ratio of sons that became roamers to sons that became territorial breeders between fathers of the two tactics using the Fisher's exact test.

DATA ANALYSIS AND STATISTICS

We used number of pups sired as our fitness estimate. This was standardized as relative fitness by dividing each male's value by the mean for the year. As individual males only breed for one spring breeding season, this was a proxy of relative lifetime reproductive success. For analyses of covariance (ANCOVA), we square-root transformed relative fitness and used single degree of freedom treatment contrasts for tests of significance of effect sizes. For comparisons over all years, we also square-root transformed body mass and centred it at zero. We fit a model with relative fitness as the dependent variable and mass, tactic and year and all interactions as explanatory variables. We used methods of model simplification after Crawley (2007), and within models we test with the t distribution differences between intercepts and slopes using contrasts. Confidence intervals were calculated in the stats package in R. The software packages SAS 9.1.3 and R 2.12.0 (R Development Core Team (2010)) were used. Data are presented as mean ± SD. Correlations were tested using Spearman correlations coefficient r_s.

Results

BODY MASS DIFFERENCES

Males differed in body mass between tactics and years (Table 2, ANOVA, $F_{5,84} = 18{\cdot}69$, $P < 0{\cdot}0001$). Territorial breeding males were heavier than roamers ($t_{1,84} = 4{\cdot}76$, $P < 0{\cdot}0001$) and philopatrics ($t_{1,84} = 7{\cdot}49$, $P < 0{\cdot}0001$). Roamers were heavier than philopatrics ($t_{1,84} = 2{\cdot}20$, $P < 0{\cdot}035$). In 2007, in the year of intermediate population density, body mass was significantly higher overall ($t_{2,84} = 3{\cdot}81$, $P < 0{\cdot}001$).

In 2005, body mass of territorial males correlated with the size of communal female groups ($r_s = 0{\cdot}60$, $P = 0{\cdot}03$, $n = 12$), but not in 2007 ($r_s = -0{\cdot}05$, $P > 0{\cdot}9$, $n = 5$). Size of communal female groups ranged from 1 to 8 in 2005 (3·5 ± 2·1 breeding females per group), and from 1 to 4 in 2007 (2·4 ± 1·1 breeding females per group).

COMPARISONS OVER ALL YEARS

Tactic, body mass and year and several of their interactions influenced relative fitness (Table 3; ANCOVA, $F_{8,81} = 18{\cdot}64$, $P < 0{\cdot}00001$). Analysis of contrasts within this model showed that the territorial breeding tactic gave higher fitness

Table 2. Body mass (mean ± SD) in grams and sample size (in brackets) of males

Population density	Year	Territorial breeders	Roamers	Philopatrics	Population mean
High	2005	56·1 ± 9·9 (12)	43·5 ± 7·9 (15)	38·8 ± 6·0 (32)	43·5 ± 9·7
Intermediate	2007	58·0 ± 8·6 (5)	54·9 ± 3·6 (8)	(0)	56·1 ± 5·9
Low	2003	(0)	47·2 ± 6·0 (18)	(0)	47·2 ± 6·0

Philopatrics born during the breeding season studied are not included.

Table 3. Parameter estimates from analyses of covariance of mass, tactic and year on fitness after model reduction

Term	Parameter estimate	Lower 95% confidence limit	Upper 95% confidence limit	t	P
Intercept	0·38	0·12	0·65	2·86	0·005
Mass	0·005	−0·25	0·26	0·037	0·971
Tactic B (vs. R)	1·57	1·11	2·03	6·87	0·00001
Tactic P (vs. R)	−0·33	−0·66	0·002	−1·98	0·051
Tactic P (vs. B)	−1·90	−2·37	−1·43	−8·01	0·000001
Year 2003 (vs. 2005)	0·10	−0·26	0·47	0·57	0·567
Year 2007 (vs. 2005)	−0·17	−0·84	0·51	−0·50	0·622
Year 2007 (vs. 2003)	−0·27	−0·94	0·39	−0·81	0·419
Mass: year 2003 (vs. 2005)	1·18	0·58	1·79	3·87	0·0002
Mass: year 2007 (vs. 2005)	0·70	−0·11	1·52	1·71	0·091
Mass: year 2007 (vs. 2003)	−0·8	−1·43	0·47	−1·00	0·319
Tactic B (vs. R): year 2007 (vs. 2005)	−1·43	−2·18	−0·67	−3·78	0·0003

Table 4. Tactic of father for all offspring for which paternity was determined

Population density	Year	Offspring	Sired by territorial breeding male	Sired by roamer	Sired by philopatric
High	2005	101	90	9	2
Intermediate	2007	98	50	48	Not present
Low	2003	98	Not present	98	Not present

than the roaming tactic and the philopatric tactic. Further, the fitness of breeders compared to roamers differed significantly between 2005 and 2007, indicating that breeders had higher fitness than roamers in 2005, but not in 2007. The effect of body mass on fitness also differed between 2005 and 2003, with mass having a stronger effect on fitness in 2003. Body mass alone, controlling for year and tactic, was not a significant predictor of fitness. There were no significant year effects. There was also no significant three-way interaction between mass, year and tactic (ANOVA, $F_{1,78} = 0{\cdot}55$, $P > 0{\cdot}46$), nor a significant interaction between mass and tactic ($F_{2,79} = 0{\cdot}20$, $P > 0{\cdot}84$); these terms were dropped from the final model presented here.

MALE FITNESS WITHIN GENERATIONS UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

Under high population density in 2005, every territorial male ($n = 12$) had some reproductive success, while 6 of 15 roamers had reproductive success. Only 2 out of 32 philopatrics present at the start of the breeding season sired offspring (Table 4), while none of the philopatrics born between August and October sired any pups. On average, territorial males sired 6·42 ± 4·32 pups, roamers 0·60 ± 1·06 pups and philopatrics 0·063 ± 0·24 pups. ANCOVA analysis of tactic and body mass and their interaction indicated that there was no significant difference between the slopes of mass against fitness for breeders compared to roamers (Fig. 1d, $t_{2,53} = -0{\cdot}70$, $P > 0{\cdot}48$) or philopatrics ($t_{2,55} = 0{\cdot}83$, $P > 0{\cdot}41$), or for roamers compared to philopatrics ($t_{2,55} = -0{\cdot}03$, $P > 0{\cdot}98$). As this interaction was not significant, it was dropped from the model for further analyses. The remaining model indicated that tactic had a highly significant effect on relative fitness ($F_{2,55} = 44{\cdot}17$, $P < 0{\cdot}00001$). Territorial breeding males had significantly higher fitness than roamers ($t_{2,55} = -7{\cdot}95$, $P < 0{\cdot}00001$) and philopatrics ($t_{2,55} = -9{\cdot}26$, $P < 0{\cdot}00001$). Roamers also had higher fitness than philopatrics ($t_{2,55} = -2{\cdot}29$, $P < 0{\cdot}03$). Body mass alone, controlling for tactic, had no effect on fitness ($t_{1,55} = 0{\cdot}04$, $P > 0{\cdot}96$).

Under intermediate population density in 2007, territorial breeding males and roamers were similarly successful (Table 4). On average, territorial males sired 9·80 ± 13·02 pups and roamers 6·00 ± 8·84 pups. Every territorial male ($n = 5$) and six of eight roamers had some reproductive success. There was no significant effect of body mass ($t_{1,9} = 1{\cdot}03$, $P > 0{\cdot}33$) or tactic ($t_{1,9} = 0{\cdot}47$, $P > 0{\cdot}65$) or their interaction ($t_{1,9} = 0{\cdot}45$, $P > 0{\cdot}66$) on relative fitness (Fig. 1e, ANCOVA, $F_{3,9} = 0{\cdot}95$, $P > 0{\cdot}45$).

While in 2005 10 of 12 territorial breeders (83%) had higher reproductive success than the most successful roamer, in 2007 only one of five territorial breeders (20%) had higher reproductive success than the best roamer. This ratio differed significantly between years ($P = 0{\cdot}03$, Fisher's exact test), indicating that the difference between territorial breeders and roamers in relative fitness between 2005 and 2007 was not simply because of low statistical power in 2007.

Under low population density in 2003, there was a significant effect of body mass on fitness (Fig 1f, linear regression, $t_{1,16} = 2{\cdot}96$, $P < 0{\cdot}01$). On average, males sired 4·56 ± 7·99 offspring with 10 (of 18; 56%) males siring no offspring at all and the most successful male siring 29 offspring with 10 different females during the entire breeding season. Five offspring were sired by 2 (of 22; 9%) young males born in September 2003 in our field site. Both of these males had left their natal group and adopted a roaming tactic. No males born in October or start of November ($n = 24$) sired any offspring, even though theoretically they could have sired offspring born in December.

TACTIC, BODY MASS AND MULTIPLE PATERNITY

The incidence of multiple paternity was high in all years: 39·3% of litters when population density was high, 25·0% of litters under intermediate population density and 54·2% of litters under low population density. Territorial breeding males lost a high percentage of paternity to other males: 39·9 ± 29·3% (range: 0·0–100·0%) of pups were sired by another male than the territorial male of the group ($n = 17$ territorial males from 2005 and 2007). Heavier males were not more successful in siring offspring with extra-group females ($r_s = 0{\cdot}18$, $n = 40$ males, $P = 0{\cdot}27$), indicating that these matings were because of female choice, not male dominance, and that females do not use body mass as a criterion for extra-group mate choice. Furthermore, for 12 females, we could compare body mass between their group male (62·1 ± 11·1 g) and the extra-group fathers of her offspring (52·3 ± 7·1 g). Extra-pair fathers were significantly lighter than group males (paired $t_{11} = 3{\cdot}409$, $P = 0{\cdot}006$). Including only the eight cases where the extra-group male was a territorial breeder, there was still a trend (paired $t_7 = 2{\cdot}168$, $P = 0{\cdot}067$).

INFLUENCE OF THE SIRE'S TACTIC ON HIS SON'S TACTIC?

For sires that were roamers, the ratio of sons that became roamers to sons that became territorial breeders was 1:13

Table 5. Last tactic shown by a male depending on the tactic of his genetic father

	Tactic son			
Tactic father	Philopatric	Roamer	Territorial breeder	SUM
Roamer	0	1	13	14
Territorial breeder	2	1	7	10
SUM	2	2	20	24

(roamers : breeders). For sires that were territorial breeders, the ratio was 1:7 (roamers : breeders). Roamers and territorial breeders did not differ in producing sons that became territorial breeders ($P > 0{\cdot}99$, Fisher's exact test; Table 5).

Discussion

Genetic parentage studies have significantly increased our knowledge of natural mating systems, but few have collected data over several years representing different generations living under different ecological conditions to provide deeper insight into its evolution. We show that three alternative male reproductive tactics yield different reproductive success for different generations. We found little evidence for a correlation between reproductive success and body mass (= status) within tactics. Our results challenge traditional definitions of strategies underlying ARTs.

Fitness differences between tactics were seen for the generation living when population density was high and females lived in communal groups. Under these conditions, defending a female group as a territorial breeding male was the best tactic, and larger males defended larger multifemale groups. While we did not measure total fitness of all males, because males might also have sired offspring outside of the study area, extra-group paternity is biased towards territorial breeders under high population density (Schradin, Schneider & Lindholm 2010), further strengthening the conclusion that breeding as a territorial breeder is the most successful tactic. This was in agreement with the definition of conditional strategy by Gross (1996) and is similar to the situation in other species, where roaming males (= wanderers in prairie voles, *Microtus ochrogaster*, Ophir *et al.* 2008) or subordinates (in meerkats, *Suricata suricatta*, Young, Spong & Clutton-Brock 2007) have significantly lower reproductive success than territorial males. As expected, being philopatric is a tactic with low reproductive success. This might explain why philopatrics have very high corticosterone levels (Schradin *et al.* 2009) as predicted for tactics where individuals are stressed because they are making the best of a bad job (Moore, Hews & Knapp 1998).

In contrast, in the generation with low population density, males expressed only one tactic, roaming, and some roamers had very high reproductive success. While under conditions of clumped female distributions males should attempt to defend groups of females, under dispersed conditions males should invest resources in searching for these dispersed females (Emlen & Oring 1977; Orians 1969; Ostfeld 1990). Accordingly, which reproductive tactic male striped mice chose depended on female distribution: when female striped mice formed groups, the largest males became the breeding males of these groups (Schradin *et al.* 2009), defending harems within a relatively small territory (Schradin & Pillay 2005b). In contrast, when females lived solitarily, males preferred to roam and occupied large home ranges, overlapping with the home ranges of several single breeding females, which they only visited for copulation (Schradin, König & Pillay 2010). If females are clumped (favouring interference competition), body mass should correlate with competitive ability and reproductive success, while when females are dispersed (favouring scramble competition), selection should favour traits such as search ability. However, this is in contrast to our present study, where body mass (independent of tactic) was important only in 2003, when males were searching for females, but not in 2005, when males defended female groups.

At intermediate population density, with a female-biased sex ratio and a ratio of single breeding females to communally breeding females of close to unity, fitness of roamers was not statistically different from that of territorial breeders. While sample size and thus the statistical power was low for 2007, we found important differences between 2007 with intermediate and 2005 with high population density: when females were living in communal groups, nearly all breeders had higher fitness than all roamers, while several roamers were better than the average breeder when many females bred solitarily. This interpretation was also supported by our comparison over years: fitness between breeders and roamers differed between 2005 and 2007, indicating that breeders were more successful than roamers when females lived communally, but not or to a much lesser extent when many females lived solitarily. The fact that males sired in total more offspring per male in 2007 than in 2005 can be explained by a change of the sex ratio from male biased in 2005 to female biased in 2007 (Table 1), which might be attributed to more males dispersing in years of lower population density, potentially leading to increased male mortality.

Our study does not demonstrate that it is population density *per se* that determines fitness consequences of male ARTs in striped mice. Fitness of the ARTs could be influenced by the relative frequency of the alternative tactics, which we know is density dependent (Schradin, König & Pillay 2010). Additionally, operant sex ratio, the ratio of solitary to communally breeding females and different ecological variables such as food availability and predation pressure could influence fitness outcomes, and some of these factors might correlate with each other and with population density. The important result of our study is that years differ significantly in fitness consequences of ARTs, and this cannot be explained adequately by existing theory.

When two tactics yield the same reproductive success, they could fit either of the definitions of alternative or mixed strategy (Gross 1996). In alternative strategies, males of alterna-

tive tactics differ genetically and are constrained to one tactic, while in mixed strategies ARTs are not the result of different genotypes, i.e. each male could follow any tactic, and the tactic is chosen based on environmental information (thus only one strategy exists; Gross 1996). It has been shown previously that male striped mice regularly switch tactics and that the same individual male can follow all three tactics during his life (Schradin *et al*. 2009), indicating that tactics are not genetically determined. In the present study, roamers were as likely to sire sons that later became territorial breeders as were territorial breeders. Our data suggest that genotype does not strongly predict which tactic will be chosen; though, genetic variation in the expression of switch-points is not ruled out. Low heritability of two tactics with equal fitness fits the definition of mixed strategies, and to our knowledge, no other empirical example fits the predictions of a mixed strategy better than our results from 2007, the year of intermediate population density.

DECISION RULES: MALE STRIPED MICE FOLLOW A SINGLE STRATEGY

A strategy defines the decision rules determining which tactic an individual chooses (Gross 1996), but these decisions rules are rarely (if ever) formulated. We propose that male striped mice follow the same decision rules in every year, i.e. they have a single strategy that can lead to 1, 2 or 3 different tactics in the population. Our study demonstrates that under most environmental conditions, the fitness consequences represent the outcome of what has been traditionally defined as a conditional strategy (as in most years roamers are smaller than territorial breeders; Schradin *et al*. 2009). However, under specific conditions, tactics might yield identical fitness, which is a characteristic of mixed strategies. Thus, our results indicate that the differentiation between conditional and mixed strategies is not an absolute one, and in many species, environmental conditions might exist under which the normally suboptimal tactic yields equal fitness to the (usually) dominant tactic, and others under which only a single tactic will prevail. We call this a single strategy indicating that all males follow the same decision rules, in contrast to alternative strategies, that consist of two or more sets of decision rules (Gross 1996). Here, we suggest decision rules for a single strategy in male striped mice representing a hypothetical model against which future data are to be tested:

1. Decision: To remain philopatric or to disperse?
- Remain a philopatric male if all females breed in communal groups defended by territorial males and your body mass is below the population mean
- Leave your natal group:
  - **a.** If the ratio of single to communally breeding females is $> 1{\cdot}0$ (Table 1).
  - **b.** If at the beginning of the breeding season your body mass is above the mean of the population (Table 2), independent of population density.

2. Decision: To become a roamer or try to become a territorial breeder?
- If all females breed solitarily $\rightarrow$ become a roaming male.
- If you find a group of communally breeding females that are not defended by a male that is larger than you $\rightarrow$ become the breeding male of this group.

This set of decision rules emphasizes the important point that being a roamer can be the result of two very different processes: (i) an active choice, because many females breed solitarily and roaming is the best tactic or as good as being a territorial breeder. (ii) Result of low competitive ability, i.e. the male chooses to disperse and would choose to become a territorial breeder, but he cannot, because all communal female groups are defended by larger males. This is similar to the situation in stoplight parrotfish (*Sparisoma viridae*) where males start with the tactic of bachelor and the availability of free territories and their relative body size determines when they can switch to the territorial tactic (Cardwell & Liley 1991). In 2003 and 2007, one alternative for roamers would have been to join a singly breeding female and to become monogamous. Defending a single female could be a strategy to reduce multiple paternities, which were more common in 2003, with 54% of litters having more than one sire. However, because of solitary foraging and the fact that female home ranges are larger when population density is lower (Schradin *et al*. 2010c), mate guarding might not be a successful tactic under these circumstances.

EVOLUTION OF SWITCH-POINTS

One main aim in the study of ARTs is to understand the regulation of switch-points, i.e. at which stage individuals switch tactics (Gross 1996). For switch-points to evolve, individuals must show genetically determined variation in their switch-points (Shuster & Wade 2003; Tomkins & Hazel 2007; Taborsky 2008a). Switch-points are typically illustrated by fitness curves, with the suboptimal tactic showing higher fitness at lower status, while the dominant tactic shows higher fitness at higher status (Fig. 1a). The switch-point is the point where these two lines cross, and individuals are expected to switch tactic when reaching this point (Gross 1996; Tomkins & Hazel 2007; Taborsky 2008a).

In striped mice, tactics depend on body mass, with the heaviest males being territorial breeders, intermediate males being roamers and the smallest males being philopatrics (Schradin *et al*. 2009; this study). According to the switch-point model, we expected a correlation between body mass and reproductive success. However, our comparison over years showed that having a higher body mass alone did not increase fitness. Accordingly, larger territorial breeders were not more successful than small territorial breeders, and larger roamers were not more successful than smaller roamers (except for 2003, when roaming was the only tactic, leading to direct male–male competition). Therefore, we found no

linear relationships between body mass and reproductive success within the different tactics and no switch-points could be determined using this approach.

Body mass influences reproductive success in many species, including those with ARTs (e.g. brook trout *Salvenius fontinalis*, Blanchfield, Ridgway & Wilson 2003; horseshoe crabs *Limulus polyphemuus*, Brockmann & Taborsky 2008; dung flies *Scathophaga stercoraria*, Pitnick *et al*. 2009). In bluegills, body condition (combining body mass and body length) but not body mass influences reproductive success (Neff & Clare 2008). Thus, the question arises whether our negative result is because of the fact that we did not measure the correct variable. We think that body mass is the best measurement because it is important in most species, and in polygynous mammals, larger males are more successful in defending multi-female groups than smaller males (Heske & Ostfeld 1990; Adrian *et al*. 2008). In striped mice, body mass strongly correlates with success in territorial encounters (Schradin 2004) and is highly correlated with male tactics (Schradin *et al*. 2009), and larger males had larger multi-female groups in the year of high population density (this study). While Neff & Clare (2008) did not find a significant effect for body mass on reproductive success in bluegills, they found a trend, and this was to be expected, as their measurement of body condition was a correlate of body mass. In contrast, the main influence of body mass we found was on what tactic was expressed. Thus, we do not believe that any other measurement of body condition would yield a significantly different result. By becoming a territorial male, males can increase reproductive success by defending paternity within their group of communally breeding females. However, this is a categorical event (breeding male yes: high fitness/no breeding male: low fitness), not an event along continuous fitness contours depending on body mass.

As our study relies on a relatively small sample size, we cannot distinguish between the two possibilities that body mass has either no or only a weak influence on male reproductive success within tactics. As theory predicts a clear effect of body mass on fitness, the missing evidence in our study needs explanation in both cases. We suggest that the reason why male reproductive success did not correlate well with body mass within tactics is female choice. While breeding males had the highest reproductive success, 40% of offspring within communal groups were sired by other males, which were often territorial males from neighbouring groups (Schradin, Schneider & Lindholm 2010). This indicates conflict between territorial males and their females, because females often preferred to mate with other males. As striped mice are solitary foragers (Schradin 2006), territorial males cannot continuously defend their females, which do encounter males from other groups during foraging (Schradin 2006). Instead, they defend territories encompassing breeding females (Schradin & Pillay 2004). Our data indicate that females often chose other males as mates in addition to their territorial male and that they did not choose large males for extra-group mating. This could explain the lack of relationship within a tactic between male mass and fitness. The factors that make a male attractive for extra-group fertilizations remain an open question.

Conclusions

Conditional strategies have been described for hundreds of species (Gross 1996) but very few studies have actually measured fitness of individual males following different reproductive tactics (exceptions are Ophir *et al*. 2008; Young, Spong & Clutton-Brock 2007), making the assessment of the status dependency of conditional strategies difficult. In fact, while many studies show a relationship between status and tactic, they typically do not conform to single switch-point predictions (Lee 2005). We find that existing theory is inadequate to describe our empirical data and we encourage theorists to review and adjust theory explaining ARTs where no underlying genetic polymorphism can be demonstrated.

Our study demonstrates three important points: (i) Despite the risk of losing a high percentage of paternity to other males (average of 40%), defending females could still be by far the best tactic. Similar results have been found in prairie voles, where territorial monogamous males lose a high percentage of paternity owing to extra-pair fertilizations, but nevertheless have three times higher reproductive success than roamers (Ophir *et al*. 2008). (ii) The differentiation between conditional and mixed strategies is not always an absolute one and can depend on environmental conditions. Instead, we propose the term 'single strategy' if all individuals follow the same (or very similar) decision rules, independent of the current fitness consequences of ARTs, which would be in contrast to alternative strategies with more than one strategy. 3. Fitness differences between tactics might be rather categorical (e.g. roamer vs. territorial breeder category) than continuous along fitness lines. We expect that many more species follow the pattern of categorical fitness differences.

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References

Adrian, O., Dekomien, G., Epplen, J.T. & Sachser, N. (2008) Body weight and rearing conditions of males, female choice and paternities in a small mammal, *Cavia aperea*. *Ethology*, **114**, 897–906.

Blanchfield, P.J., Ridgway, M.S. & Wilson, C.C. (2003) Breeding success of male brook trout (*Salvelinus fontinalis*) in the wild. *Molecular Ecology*, **12**, 2417–2428.

Brockmann, H.J. & Taborsky, M. (2008) Alternative reproductive tactics and the evolution of alternative allocation phenotypes. *Alternative Reproductive Tactics* (eds R.F. Oliveira, M. Taborsky & H.J. Brockmann), pp. 25–51. Cambridge University Press, Cambridge.

Brooks, P.M. (1982) Aspects of the reproduction, growth and development of the four-striped mouse, *Rhabdomys pumilio* (Sparrman, 1784). *Mammalia*, **46**, 53–64.

Cardwell, J.R. & Liley, N.R. (1991) Androgen control of social status in males of a wild population of stoplight parrotfish, *Sparisoma viridae* (Scaridae). *Hormones and Behavior*, **25**, 1–18.

Crawley, M.J. (2007) *The R Book*. John Wiley & Sons, Ltd., Chichester.

Dawkins, R. (1980) Good strategy or evolutionary stable strategy?. *Sociobiology: Beyond Nature/Nurture* (eds G.W. Barlow & J. Silverberg), pp. 331–367. Westview, Boulder, CO.

Emlen, S.T. & Oring, L.W. (1977) Ecology, secual selection, and the evolution of mating systems. *Science*, **197**, 215–223.

Gross, M.R. (1996) Alternative reproductive strategies and tactics: diversity within the sexes. *Trends in Ecology and Evolution*, **11**, 92–98.

Heske, E.J. & Ostfeld, R.S. (1990) Sexual dimorphism in size, relative size of testes, and mating systems in North American microtine rodents. *Journal of Mammalogy*, **71**, 510–519.

Kalinowski, S.T., Taper, M.L. & Marschall, T.C. (2007) Revising how the computer program Cervus accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, **16**, 1099–1106.

Krebs, J.R. & Davies, N.B. (1993) *An Introduction to Behavioural Ecology*, 3rd edn. Blackwell Science Ltd, Oxford.

Lane, J.E., Boutin, S., Gunn, M.R. & Coltman, D.W. (2009) Sexually selected behaviour: red squirrel males search for reproductive success. *Journal of Anima Ecology*, **78**, 296–304.

Lank, D.B., Smith, C.M., Hanotte, O., Burke, T. & Cooke, F. (1995) Genetic polymorphism for alternative mating behaviour in lekking male ruff *Philomachus pugnax*. *Nature*, **378**, 59–62.

Lee, J.S.F. (2005) Alternative reproductive tactics and status-dependent selection. *Behavioral Ecology*, **16**, 566–570.

Moore, M.C., Hews, D.K. & Knapp, R. (1998) Hormonal control and evolution of alternative male phenotypes: generalizations of models for sexual differentiation. *American Zoologist*, **38**, 133–151.

Neff, B.D. & Clare, E.L. (2008) Temporal variation in cuckoldry and paternity in two sunfish species (*Lepomis spp.*) with alternative reproductive tactics. *Canadian Journal of Zoology*, **86**, 92–98.

Ophir, A.G., Phelps, S.M., Sorin, A.B. & Wolff, J.O. (2008) Social but not genetic monogamy is associated with greater breeding success in prairie voles. *Animal Behaviour*, **75**, 1143–1154.

Orians, G.H. (1969) On the evolution of mating systems in birds and mammals. *The American Naturalist*, **103**, 589–603.

Ostfeld, R.S. (1990) The ecology of territoriality in small mammals. *Trends in Ecology and Evolution*, **5**, 411–415.

Pitnick, S., Henn, K.R.H., Maheux, S.D., Higginson, D.M., Hurtado-Gonzales, J.L., Manier, M.K., Berben, K.S., Guptill, C. & Uy, J.A.C. (2009) Size-dependent alternative male mating tactics in the yellow dung fly, *Scathophaga stercoraria*. *Proceedings of the Royal Society B: Biological Sciences*, **276**, 3229–3237.

R Development Core Team (2010) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. Vienna, Austria. Available at: http://www.R-project.org/.

Schradin, C. (2004) Territorial defense in a group living solitary forager: who, where, against whom? *Behavioral Ecology and Sociobiology*, **55**, 439–446.

Schradin, C., König, B. & Pillay, N. (2010) Reproductive competition, not population density, drives sociality in African striped mice! *Journal of Animal Ecology*, **79**, 515–521.

Schradin, C. & Pillay, N. (2004) The striped mouse (*Rhabdomys pumilio*) from the succulent karoo of South Africa: a territorial group living solitary forager with communal breeding and helpers at the nest. *Journal of Comparative Psychology*, **118**, 37–47.

Schradin, C. & Pillay, N. (2005a) Demography of the striped mouse (*Rhabdomys pumilio*) in the succulent karoo. *Mammalian Biology*, **70**, 84–92.

Schradin, C. & Pillay, N. (2005b) Intraspecific variation in the spatial and social organization of the African striped mouse. *Journal of Mammalogy*, **86**, 99–107.

Schradin, C. & Pillay, N. (2006) Female striped mice (*Rhabdomys pumilio*) change their home ranges in response to seasonal variation in food availability. *Behavioral Ecology*, **17**, 452–458.

Schradin, C., Schneider, C. & Lindholm, A.K. (2010) The nasty neighbour in the striped mouse (*Rhabdomys pumilio*) steals paternity and elicits aggression. *Frontiers in Zoology*, **7**, 19.

Schradin, C., Scantlebury, M., Pillay, N. & König, B. (2009) Testosterone levels in dominant sociable males are lower than in solitary roamers: physiological differences between three male reproductive tactics in a sociably flexible mammal. *American Naturalist*, **173**, 376–388.

Schradin, C., Schmohl, G., Rödel, H.G., Schoepf, I., Treffler, S.M., Brenner, J., Bleeker, M., Schubert, M., König, B. & Pillay, N. (2010c) Female home range size is regulated by resource distribution and intraspecific competition: a long-term field study. *Animal Behaviour*, **79**, 195–203.

Shuster, S.M. & Sassaman, C. (1997) Genetic interaction between male mating strategy and sex ratio in a marine isopod. *Nature*, **388**, 373–376.

Shuster, S.M. & Wade, M.J. (2003) *Mating Systems and Strategies*. Princeton University Press, Princeton.

Solmsen, N., Johannesen, J. & Schradin, C. (2011) Highly asymmetric fine-scale genetic structure between sexes of African striped mice and indication for condition dependent alternative male dispersal tactics. *Molecular Ecology*. DOI: 10.1111/j.1365-294X.2011.05042.x.

Taborsky, M. (2008a) Alternative reproductive tactics in fish. *Alternative Reproductive Tactics* (eds R.F. Oliveira, M. Taborsky & H.J. Brockmann), pp. 251–299. Cambridge University Press, Cambridge.

Taborsky, M. (2008b) The use of theory in behavioral research. *Ethology*, **114**, 1–6.

Taborsky, M., Oliveira, R.F. & Brockmann, H.J. (2008) The evolution of alternative reproductive tactics: concepts and questions. *Alternative Reproductive Tactics* (eds R.F. Oliveira, M. Taborsky & H.J. Brockmann), pp. 1–22. Cambridge University Press, Cambridge.

Teschke, M., Mukabayire, O., Wiehe, T. & Tautz, D. (2008) Identification of selective sweeps in closely related populations of the house mouse based on microsatellite scans. *Genetics*, **180**, 1537–1545.

Tomkins, J.L. & Hazel, W. (2007) The status of the conditional evolutionary stable strategy. *Trends in Ecology and Evolution*, **22**, 522–528.

Young, A.J., Spong, G. & Clutton-Brock, T. (2007) Subordinate male meerkats prospect for extra-group paternity: alternative reproductive tactics in a cooperative mammal. *Proceedings of the Royal Society of London. Series B*, **274**, 1603–1609.

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Chapter 4

Social flexibility and social evolution in mammals: a case study of the African striped mouse (*Rhabdomys pumilio*)

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INVITED REVIEW

Social flexibility and social evolution in mammals: a case study of the African striped mouse (*Rhabdomys pumilio*)

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Abstract

Environmental change poses challenges to many organisms. The resilience of a species to such change depends on its ability to respond adaptively. Social flexibility is such an adaptive response, whereby individuals of both sexes change their reproductive tactics facultatively in response to fluctuating environmental conditions, leading to changes in the social system. Social flexibility focuses on individual flexibility, and provides a unique opportunity to study both the ultimate and proximate causes of sociality by comparing between solitary and group-living individuals of the same population: why do animals form groups and how is group-living regulated by the environment and the neuro-endocrine system? These key questions have been studied for the past ten years in the striped mouse *Rhabdomys pumilio*. High population density favours philopatry and group-living, while reproductive competition favours dispersal and solitary-living. Studies of genetic parentage reveal that relative fitness of alternative reproductive tactics depends on the prevailing environment. Tactics have different fitness under constrained ecological conditions, when competitive ability is important. Under conditions with relaxed ecological constraints, alternative tactics can yield equal fitness. Both male and female striped mice display alternative reproductive tactics based on a single strategy, i.e. all individuals follow the same decision rules. These changes are regulated by endocrine mechanisms. Social flexibility is regarded as an adaptation to unpredictably changing environments, selecting for high phenotypic flexibility based on a broad reaction norm, not on genetic polymorphism for specific tactics.

Keywords: communal breeding, mate choice, paternal care, prolactin, prairie vole, testosterone

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Social flexibility

Behavioural ecology seeks to understand how animals survive and reproduce in their natural environment. However, the environment is not static, but changes in predictable and unpredictable ways (Wingfield, 2003). Long-term field studies are needed to understand individual responses to changing environments and how these may affect the evolution of social behaviour (Clutton-Brock & Sheldon 2010). Natural environments are predicted to change faster in the future due to anthropogenic induced climate change (Friedlingstein 2008), testing the limits of behavioural adaptation and resilience of natural populations.

The term *social flexibility* is generally used to describe modifications of individual social behaviours, but its usage differs among authors. A search in the ISI Web of Science for the term 'social flexibility' revealed 276 publications in the field of Zoology for the period 1900 to 2010. Most papers were about 'behavioural flexibility' of non-social behaviours, only 84 papers were about flexibility in social behaviour and no clear difference was made between 'social flexibility', 'intra-specific variation in social behaviour', and 'alternative reproductive tactics'. Used in such a way, the term 'social flexibility' simply means that social behaviour is flexible, which is true for nearly all social behaviours of all species.

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We developed the concept of social flexibility from our long-term field studies on the striped mouse (*Rhabdomys pumilio*; Fig. 1). Here, we define the concept of social flexibility, in which the social organization (the composition of groups) within a population changes facultatively as a function of individuals of both sexes changing their reproductive and social tactics in response to changing environmental conditions (Box 1). As a consequence, the entire social system (comprising of the social organization, the mating system, and the social structure of a population; Kappeler & van Schaik 2002) can change, although this is not a pre-requisite for social flexibility (see examples below).

Social plasticity is a similar term, but can include examples where during ontogenetic processes one of several alternative tactics is developed and maintained during adulthood. Social flexibility comprises the cases of social plasticity in which adult individuals can switch back and forth between tactics. Other similar terms have been used, such as 'intra-specific variation social systems' (Lott 1991), which in contrast to social flexibility could also be a result of genetic differences between populations. The term 'flexible social structure' (Randall *et al.* 2005) describes a case where due to low population density (absence of surviving female relatives) individuals might have to live solitarily. To demonstrate social flexibility in the way we define it, its important to show that individuals of both sexes (not only males like in many species with alternative reproductive tactics) can chose their social organization, for example to live solitarily or to live in groups. In contrast to this, one simple explanation for solitary versus group-living could be differences in population density. Individuals may be constrained to live solitarily when population density is very low, and constrained to be group-living when population density is very high, without giving individuals a choice of social organization. However, there is little empirical support for this. Obligate social species form groups even under very low population densities, for example lions in the Kalahari (Bothma & Walker 1999). On the other hand, some species like whistling rats (*Parotomys brantsii*) live solitarily even under very high population densities (Jackson 1999).

Social flexibility can help to understand the evolution of social systems, since social flexibility results from a complex interaction between individuals and their environment, causing physiological and behavioural reactions (Lott 1991). Understanding social flexibility is an important research topic, especially when we consider that our own species is characterized by high social flexibility with monogamous, polygynous and polyandrous societies, and different individuals following alternative tactics within societies.

Box 1 Social flexibility

Social system: The social system of a species comprises the social organization (the composition of groups, e.g. solitary or family groups), the mating system, and the social structure (describing which individuals interact with each other; Kappeler & van Schaik 2002).

Intra-specific variation in social systems: This term was introduced by Lott (1984;1991) who did not provide a precise definition, but used the term very broadly for variation in group size, territoriality, dominance status, parental care, and mating systems. Intra-specific variation can occur between populations and might have a genetic basis, so that individuals following alternative tactics differ genetically.

Social flexibility: Unlike intra-specific variation, social flexibility focuses on individual flexibility. It describes the phenomenon that individuals change their social and reproductive tactic, modify their interactions with other individuals (social structure), with whom they mate (mating system), and consequently the composition of groups (social organization). If this occurs in both sexes, it influences the entire social system of the population, for example from solitary living to single family groups (pair plus offspring) to extended family groups. Within a population, often two forms of social organization might exist, e.g. both solitary and group-living individuals or monogamous and polygynous groups. We predict social flexibility to be an adaptation to unpredictably changing environments, selecting for high phenotypic flexibility that is based on a broad reaction norm, not on genetic polymorphisms for specific tactics.

Definition of social flexibility: Both males and females have alternative reproductive and social tactics based on a single strategy (all individuals have the same decision rules). Depending on environmental as well as individual conditions (e.g. social status, competitive ability) individuals choose to live solitarily, in small or large social groups (though not all individuals might be able to live under the condition of their choise; for an example see Box 3). Consequently, the social system of a species or population can change as a function of individuals changing their tactics.

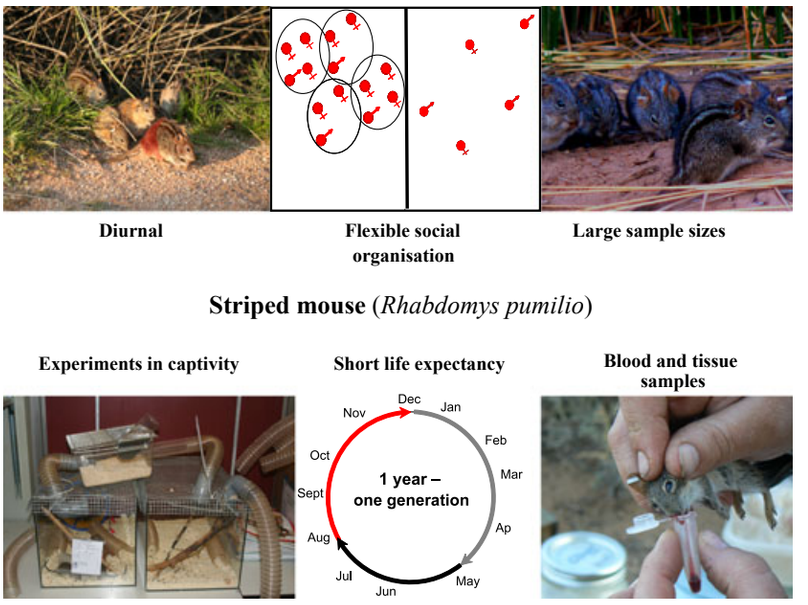

Fig. 1 Advantages of the striped mouse as a study species (from top left to bottom right). 1. It is diurnal, inhabits an open habitat and easily habituates to the presence of observers, making direct behavioural observations possible in the field. 2. High social flexibility, ranging from extended family groups (left) to solitary living (right) in the same population. 3. Large sample sizes. 4. Can be studied in captivity. 5. Short generation time (red: breeding season in spring; grey: hot dry season; black: cold rainy season). 6. Samples for hormone measurements and genetic analyses can be easily collected. Blood samples are taken from a sub-lingual vein, a method less harmful than traditional methods of blood sampling used in mice (Heimann *et al.* 2009).

Social flexibility in the African striped mouse

First studies indicated that the striped mouse of the Succulent Karoo is best characterized as a territorial, group-living, solitary forager with communal breeding, paternal care and helpers at the nest (Schradin & Pillay 2004). Groups of close kin consist of up to 30 adult individuals: a single breeding male, two to four communally breeding females and their adult offspring of both sexes that act as helpers at the nest (Schradin & Pillay 2004). Groups of striped mice sleep together in one nest and share a territory which they defend against neighbouring groups (Schradin 2004). Individuals from the same group forage solitarily during the day but bask and interact with each other amicably in front of their nest at dawn and at dusk (Schradin *et al.* 2007). After a severe drought in 2003, population density declined significantly from 70 mice/ha to 2 mice/ha, but striped mice still formed groups, though these huddling groups consisted of non-related individuals (Schradin *et al.* 2006). With the onset of the breeding season, all individuals switched from group- to solitary-living, but they became group-living again at the end of the breeding season, when their adult offspring remained philopatric (Schradin *et al.* 2010a). Thus, individuals of both sexes switched from group- to solitary-living and back to group-living, indicating high social flexibility (Schradin *et al.* 2010a), offering a unique opportunity to study ultimate and proximate causes of sociality (Box 2).

Social flexibility occurs in many taxa

Intra-specific variation in the social system has been observed in more than a hundred vertebrate species (Lott 1991), but in most cases it is not known whether this is due to genetic differences between individuals (or populations), or due to social flexibility, i.e. flexibility in the social behaviour of individuals. Indication exists also for invertebrates such as the burying beetle (*Necrophorus vespilloides*). In this species both males and females have alternative reproductive tactics: males can attract females via pheromones either after they searched for and found a carcass (searching tactic) or without having access to a carcass (pure pheromone tactic), or they act as satellite males at carcasses defended by larger males (Eggert, 1992). Each male can use all three tactics (Eggert, 1992), indicating behavioural flexibility. Females either have a carcass for themselves or with one other

female, which could be either a subordinate female (called parasite), or a female of equal status (joint or communal breeding; Müller *et al.*, 2006). The main factors influencing the resulting social system are differences in body mass between individuals and the availability of food resources, i.e. carcass size. Thus, in burying beetles females may breed solitarily (having previously mated with a pheromone secreting male), in monogamous bi-parental pairs, as parasitic subordinate females, or in communally breeding groups with two females and one male sharing a carcass (Eggert and Müller 2000; Müller *et al.* 2006). This system fits our definition of social flexibility where the social system changes as a function of individuals of both sexes changing their tactics depending on environmental conditions.

In several bird species, social flexibility is represented by switching from breeding in monogamous pairs to single families to extended family groups, which has been used to study cooperative breeding (Emlen 1997; Koenig *et al.* 1992). In dunnocks *(Prunella modularis)*, social organization includes monogamous pairs, polyandric, polygynous or polygynandrous groups. Females defend exclusive territories, with territory size depending on density of competing females and habitat quality. A single male or groups of unrelated males (depending on competitive ability) defend territories that overlap those of one or more females. Females mate with all resident males, resulting in a mating system that ranges from monogamy to polygynandry. Studies on dunnocks have helped to understand the evolution of sexual conflict, mating systems, parental effort and life histories (Davies 1992).

Solitary-living is quite rare in birds, but occurs in many mammalian species. Social flexibility seems to be especially prevalent in short lived rodents that need to quickly respond to changing environmental conditions (Lott 1991). Among rodents, several species that are solitary during summer form huddling groups during winter (Madison *et al.* 1984; Webster & Brooks 1981), which function to save energy (Canals *et al.* 1989). In the great gerbil (*Rhombomys opimus*) the social organization comprises solitary living, pair-living and large social groups (Randall *et al.* 2005). Great gerbil males have reproductive tactics similar to male striped mice, being philopatric, a solitary roamer, or to be the breeding male of a group. Females can also be either solitary or group-living, but it is not clear whether this reflects individual choice or is the result of availability of surviving female kin, as population density is one of the main factors influencing sociality in great gerbils (Randall *et al.* 2005).

Box 2 Social flexibility in African striped mice as a tool for studying social behaviour

Social flexibility in the striped mouse offers the opportunity to study proximate and ultimate aspects of social behaviour by comparing solitary and sociable individuals of the same population and by studying individuals changing their tactics.

1. **Benefits of group living**: individuals sleeping in huddling groups spend 25% less energy than solitary individuals (Scantlebury *et al.* 2006).
2. **Reasons for group *versus* solitary-living:** reproductive competition during the breeding season leads to solitary living when vacant territories are available, while ecological constraints force individuals to remain in communal groups (Schradin *et al.* 2010a).
3. **Communal breeding:** allo-parental care is shown by breeding females towards the pups of other females in the group (Schradin 2006; Schubert *et al.* 2009), but female infanticide induces high costs. In breeding experiments, males with larger testes were more successful in fertilizing several females synchronously than males with smaller testes (i.e. larger testes reduced the risk of sperm depletion; Schradin *et al.* 2009a). Synchronous mating leads to synchronous births, reduced risk of infanticide (females cannot discriminate between their own and unrelated pups before weaning; Pillay 2000)§ and is thus important for the evolution of communal breeding
4. **Paternal care:** paternal care leads to better pup development due to improved thermoregulation (Schradin & Pillay 2005). Costs of paternal care (missed mating opportunities) are low when males defend several females breeding in communal groups (Schradin & Lindholm 2011).
5. **Extra group paternity:** when population density is high, being a breeding male appears to be the most successful tactic in spite of a ~30% frequency of extra group paternity (Schradin & Lindholm 2011).
6. **Alternative reproductive tactics:** males adopting three alternative reproductive tactics differ in prolactin, corticosterone and testosterone levels (Schradin *et al.* 2009b). By measuring the fitness consequences of each tactic, the distinction between mixed and conditional strategies was not definitive, and instead the term ''single strategy'' was suggested (see Box 3; Schradin & Lindholm 2011).
7. **Dispersal tactics:** male striped mice have status dependent alternative dispersal tactics (Solmsen *et al.* 2011).

In prairie voles *(Microtus ochrogaster)*, individuals of the same population can be socially monogamous, solitary promiscuous or polygynous (McGuire and Getz 1998). While this species has been used as a model to study monogamy (Carter & Getz 1993; Young 2009), its social system is very similar to the one of the striped mouse, as it displays different reproductive tactics in both sexes. Males can be either philopatric, solitary wanderers (=roamers), or resident breeders at a nest with one or more breeding females. Females can be either philopatric, solitary breeders, single or plural breeders within social groups (monogamous pairs or communally breeding groups). This social flexibility has been used as a tool to understand the ecological (Lucia *et al.* 2008), evolutionary (Ophir *et al.* 2008b; Mabry et al. 2011), neuro-endocrine (Cushing *et al.* 2004; Young & Wang 2004) and genetic (Ophir *et al.* 2008a; Mabry et al. 2011; Solomon *et al.* 2009) bases of group-living and social bonding.

The house mouse (*Mus domesticus*) is another socially flexible species with alternative reproductive tactics in both sexes (Beery *et al.* 2008). Males can be subordinate philopatrics, monogamously mated paternal males (Elwood & Kennedy 1991), roaming males, or polygynous males defending a territory with a group of cooperatively breeding females. Similarly, females can be non-breeding subordinate philopatrics, or solitary or cooperative breeders (Latham & Mason 2004). Individuals can change their tactics. The factors influencing these decisions are at the moment not well understood, but population density and individual competitive ability are probably important. As a result of these individual tactics, the social system of a population can be primarily either solitary, small family groups or extended family groups with communal breeding.

Reasons for group *vs.* solitary living and the regulation of social flexibility

Group-living can yield significant benefits, as revealed by studies comparing solitary with sociable species (Pulliam & Caraco 1984; Krebs & Davies 1993) or by studying groups of different sizes in obligate group-living species (Dunbar 2002; Taborsky 1984; Clutton-Brock 2005). However, in the former case, phylogeny may confound comparisons between species, whereas in the latter case, it is difficult to determine which benefits of group-living have led to its evolution and which became prevalent only *after* complex social groups had evolved. Social flexibility avoids such confounding effects since comparisons are made between solitary and group living individuals of the same population and within individuals switching their tactics.

Group-living striped mice are solitary foragers and do not share information about food locations in their territory (Schradin 2007). However, they benefit from sharing a nest, which might lead to increased vigilance against nocturnal predators (Schradin 2005). Huddling yields significant energy savings: striped mice sleeping alone spend about 25% more energy than striped mice sleeping in huddling groups (Scantlebury *et al.* 2006). Thermoregulatory benefits of huddling are probably the main reason for group-living in striped mice and many other small mammals (Canals *et al.* 1989).

Group-living also induces costs, explaining why individuals disperse from their natal group and become solitary. Two hypotheses have been proposed to explain this change in social system. 1. The ecological constraints (habitat saturation) hypothesis predicts that individuals remain in their natal group when no opportunities for independent breeding exist, or when the costs of dispersal are higher than the costs of remaining philopatric (Koenig *et al.* 1992; Emlen 1997). 2. The reproductive competition hypothesis predicts that individuals leave their social group and breed solitarily to avoid competition for reproduction within groups, often expressed as infanticide or sexual suppression (Dobson 1982; Emlen 1997; Clutton-Brock 2005). Benefits of group-living promote philopatry, ecological constraints increase costs of dispersal also promoting philopatry, while reproductive competition increases costs of remaining philopatric. The net costs or benefits of these different factors will ultimately determine whether an individual remains philopatric or disperses.

Ecological conditions can be easily manipulated experimentally by removing individuals and thus changing population density. Several experimental studies provide support for the ecological constraints hypothesis (Pruett-Jones & Lewis 1990; Komdeur 1992; Bergmüller *et al.* 2005; Lucia *et al.* 2008). In contrast, experimental manipulation of reproductive competition is difficult, even though we know that reproductive competition has significant costs and can lead to reproductive skew (Kokko 2003).

Field data collected over eight years indicated that striped mice of both sexes were group-living during the breeding season when population density was high, but solitary living when population density was low (due to high predation pressure or low food availability), supporting the ecological constraints hypothesis (Fig. 2; Schradin *et al.* 2010a). Reproductive competition is prevalent during the breeding season in the form of reproductive suppression in males (Schradin *et al.* 2009c) and female infanticide (Schradin *et al.* 2010a). This can explain why females leave the communal group and start breeding alone when free territories become available. Communal groups often break apart at the start of the breeding season if population density is low. Female distribution seems to determine male reproductive

tactics: males join existing groups of communally breeding females, but if females are singly dispersed, males adopt a solitary roaming tactic (Schradin *et al.* 2010a). To study the effect of reproductive competition on sociality, comparisons were made of the social system between periods with and without reproductive competition. After the breeding season, in the absence of reproductive competition, the positive correlation between population density and percentage of group-living striped mice was absent and striped mice lived in groups, even under very low population densities (Fig. 2). Striped mice thus prefer to live in groups and gain benefits of group living outside the breeding season when costs of reproductive competition are absent. During the breeding season, however, they avoid reproductive competition by living solitarily when free territories are available, but are forced to live in groups when population density is high.

The degree of reproductive competition in a group can be measured by reproductive skew, and a low level of reproductive competition is believed to favour group-living (Ragsdale, 1999; Kokko, 2003; Clutton-Brock, 2005). A few previous studies have reported that groups might grow larger after the breeding season when reproductive competition is absent (Kraaijeveld & Dickinson 2001), and many bird and fish species become non territorial after the breeding season and form anonymous flocks or swarms in which members do not establish individualized relationships (Krause & Ruxton 2002). However, these species are not solitary during the breeding season, but instead form smaller social groups. Few other studies have provided comparisons between solitary-living and group-living individuals of the same species and population (but see Wcislo *et al.*, 1997; Randall *et al.* 2005; Purcell & Aviles, 2007). While reproductive competition has been observed in many taxa (Koenig et al. 1995; Faulkes & Bennett 2001; Wingfield & Sapolsky 2003; Clutton-Brock, 2005;), our study was the first to provide empirical evidence that reproductive competition can lead to solitary-living.

Alternative reproductive tactics

In species with social flexibility individuals of both sexes are able to switch between alternative reproductive tactics (ARTs). The phenomenon of alternative reproductive tactics has been analysed by game theory, where a tactic refers to a specific behaviour resulting from individual decision rules, so-called strategies (Gross 1994; Tomkins & Hazel 2007; Box 3). Adult female striped mice have the following ARTs: they can remain in their natal group and breed communally, or leave the group and start solitary breeding (Schradin *et al.* 2010a). The different factors influencing which tactic a female chooses and the resulting consequences are currently under research. ARTs are better understood in male striped mice which can follow one of three tactics (Fig. 3; Schradin *et al.* 2009b): (1) Philopatric males that might sneak copulations with females from neighbouring groups, but do not breed with the females of their natal group. These males show allo-parental care. (2) Solitary living roaming males that attempt to copulate with single breeding females or with females from communal groups, and do not show paternal care. (3) Territorial breeding males that defend groups of communally breeding females. These males show high levels of paternal care. Breeding males always originate from other groups, because males cannot obtain the breeding position in their natal group.

Box 3 Tactic and strategy

Tactic: the behaviour of an individual. For example the tactic of defending a group of communally breeding females *or* the tactic of solitary roaming.

Strategies: a strategy describes the decision rules of an individual, determining which tactic it will follow. Gross (1996) defined three categories of strategies:

1. Alternative strategies: genetically polymorphic, based on frequency dependent selection. Different tactics yield the same average fitness. Examples are male ruffs *(Philomachus pugnax)* and males of the isopod *Paracerceis sculpta* (Lank *et al.*, 1995; Shuster and Sassaman, 1997).

2. Mixed strategies: genetically monomorphic, based on frequency dependent selection. Different tactics yield the same average fitness. No good empirical examples exist (Gross 1996). Mixed strategies have also been characterized by a probabilistic basis, i.e. a probability x to play tactic X and probability 1-x to play tactic Y (Tomkins & Hazel 2007).

3. Conditional strategies: genetically monomorphic, based on status dependent selection. Different tactics yield different fitness. The tactic that an individual chooses depends on its competitive abilities. The most competitive individuals follow the tactic that yields the greatest fitness payoff, called the bourgeois tactic. Less competitive males (often called sneaker or satellite), that are often smaller and younger than the bourgeois males, make the best of a bad job (Dawkins 1980), following a tactic with

lower fitness that is still better than no reproductive success at all. These males change to the bourgeois tactic when they grow larger. Many examples exist in both vertebrates and invertebrates (Gross 1996).

Criticism of Gross definitions: The definition by Gross (1996) has been criticized on theoretical grounds because of its focus on genetic polymorphism *versus* genetic monomorphism: even if different animals follow very similar decisions rules and show flexibility, i.e. they can change between tactics, they still might differ genetically, e.g. in their decision when to switch tactics (Shuster & Wade 2003; Taborsky *et al.* 2008; Tomkins & Hazel 2007). Tomkins & Hazel (2007) further concluded that mathematical models can neither prove that fitness must be unequal (as was proposed by Gross 1996) nor that fitness must be equal (as was proposed by Shuster & Wade 2003). Our empirical study contributes data to this criticism.

Single strategy: this is a new term introduced by Schradin & Lindholm (2011) to replace the terms mixed and conditional strategy, which differ mainly in the predicted fitness consequences (equal *versus* different fitness payoffs for alternative tactics). Single strategies are not based on genetic polymorphisms, but all individuals follow the same or very similar decision rules when choosing a tactic. Individuals have plastic tactics, which means they can switch tactics, often repeatedly. Environmental conditions determine whether the different tactics yield similar or different fitness.

Unfortunately, studies on strategies underlying ARTs typically lack a formulation of the decision rules. Formulating the strategy for male striped mice has helped to explain why a single strategy can yield different fitness payoffs (Schradin & Lindholm 2011):

1. Decision: remain philopatric or disperse?

I. Remain a philopatric male if all females breed in communal groups defended by breeding males and your body mass is below the population mean.

II. Disperse from natal group:

a. If population density is low and more singly than communally breeding females are present in the population.

b. If your body mass at the beginning of the breeding season is above the population mean, independent of population density.

2. Decision: become a roamer or attempt to be a territorial breeder?

I. Become a roaming male if all females breed solitarily.

II. Become a breeding male if you find a group of communally breeding females that are not defended by a male that is larger than you.

By formulating the single strategy (=decision rules) for striped mice, it becomes evident that fitness outcomes depend on the prevailing environment. To become a roamer can be the result of a male choosing to disperse and to roam, as the tactic yields high fitness. Alternatively, it can be the result of a male which chooses to disperse and attempts to become a territorial breeder, but was not competitive enough. In the latter case, being a roamer would be a sub-optimal tactic.

To understand the evolution of male ARTs in striped mice, the first step was to determine their strategy. Traditionally, behavioural studies distinguish among three strategies for ARTs. In alternative strategies, males of different tactics are genetically polymorphic but have the same fitness (Gross 1996). Male striped mice can switch their tactic within their lifetime (Fig. 3), which is in contrast to alternative strategies based on genetic polymorphisms. To distinguish between mixed or con-

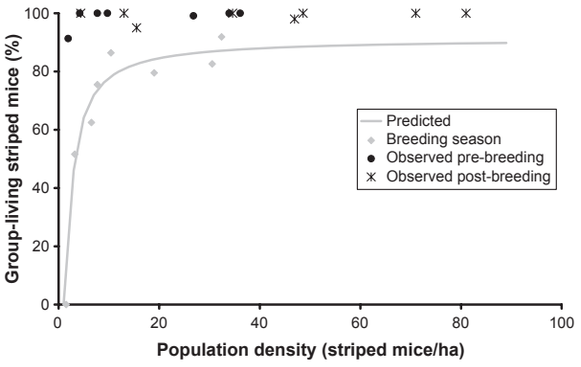

Fig. 2 Effect of population density on social organization during the breeding and the non-breeding season. Hyperbolic regression curve of the relationship between population density and percentage of group-living striped mice during the breeding season (grey diamonds; best fit, $P < 0.001$). Crosses give the observed percentages for eight post-breeding seasons and points for seven pre-breeding seasons. Figure from (Schradin *et al.* 2010a).

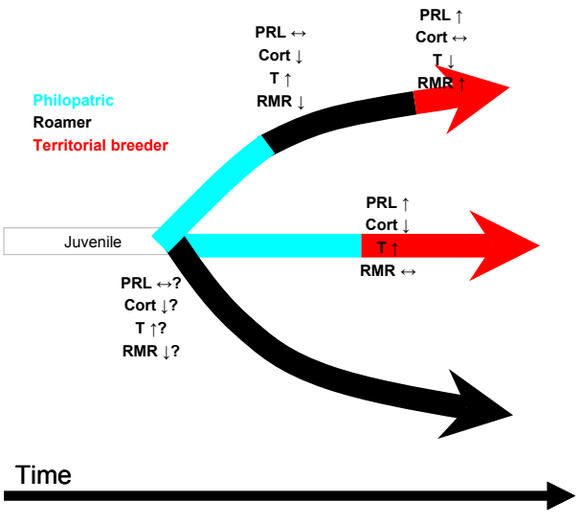

Fig. 3 Alternative male reproductive tactics in striped mice and correlated physiological changes. When reaching adulthood, male striped mice in the Succulent Karoo typically remain philopatric in their natal group (top and middle arrows). In the subsequent breeding season, philopatrics can become roamers first (black, top arrow), which is associated with an increase in testosterone (T) and a decrease in corticosterone (Cort) and resting metabolic rate (RMR), before becoming territorial breeders and experiencing an increase in prolactin (PRL) and RMR and a decrease in T. If philopatrics gain sufficient body mass during the dry season and winter, they can directly become territorial breeders the subsequent breeding season (middle arrow), which is associated with a decrease in Cort and an increase in T and PRL. If population density is very low, males can become roamers in the season of their birth, which is predicted to be associated with an increase in T and a decrease in Cort and RMR. ↓ decrease; ↑: increase; ↔: stays the same; ?: predicted change. Figure from (Schradin *et al.* 2009b).

ditional strategies, one needs to know whether the males adopting different tactics differ in competitive ability, which would indicate status dependent selection and a conditional strategy (Box 3). For conditional strategies, males with the highest competitive ability follow the dominant bourgeois tactic with much greater fitness than males with a low competitive ability doing the best of a bad job. In contrast, under mixed strategies, alternative tactics yield on average the same fitness.

Males adopting different tactics differed significantly in body weight, with territorial breeders being the heaviest males, philopatrics being the smallest males and roamers occupying an intermediate position (Schradin *et al.* 2009b). Furthermore, philopatrics can switch to roamers when they increase body mass, and roamers can switch into breeders when they become heavier (Schradin *et al.* 2009b). As heavier males are better in winning territorial encounters (Schradin 2004), these results indicated that male striped mice follow a conditional strategy. This was supported in a study of the paternities of 125 pups from 10 groups using nine microsatellite loci. The reproductive success of territorial breeding males was ten times higher than that of solitary roaming males, and a hundred times higher than that of philopatrics (Fig. 4A; Schradin & Lindholm 2011). This was one of the first studies using molecular markers to measure fitness consequences of male ARTs, with similar results in other species, where roaming males (=wanderers in prairie voles, *Microtus ochrogaster*, Ophir *et al.* 2008) or subordinates (in meerkats, *Suricata suricatta*, Young *et. al.* 2007) have significantly lower reproductive success.

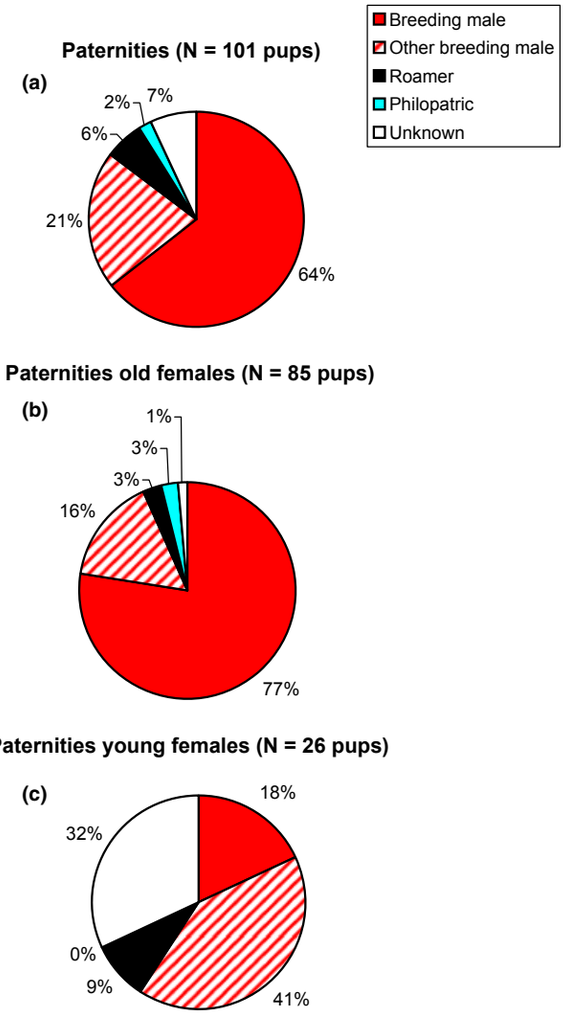

Fig. 4 Paternity within groups by the breeding male of the group, neighbouring breeding males, roamers, philopatrics and unknown males during a year with high population density, for (a) entire groups, (b) pups from old breeding females born the previous breeding season, (c) pups from young philopatric females born during the same breeding season. Figure from (Schradin *et al.* 2010b).

Genetic parentage studies have significantly increased our knowledge of natural mating systems, but few have collected data for different generations. The above-mentioned study was performed in a year of high population density, i.e. when ecological constraints were high. We repeated the study, measuring male fitness for another generation living under intermediate population density (with relaxed ecological constraints). In this generation, breeding males and roamers had similar body mass and similar fitness (Schradin & Lindholm 2011). These results were in agreement with the theory of mixed strategies and thus in contrast to the conclusion of conditional strategy derived from the results obtained from the generation living under high population density.

Do male striped mice follow a mixed or a conditional strategy? Conditional strategies are believed to arise from status dependent selection, predicting a positive correlation between fitness and status (status is typically measured as the condition of the individual). Status is often measured as body mass, a correlate of competitive ability. In theory, the slopes of the predicted fitness lines differ between tactics, leading to switch points at which individuals should switch tactics (see graphs in Taborsky *et al*. 2008). However, we did not find correlations between body mass and fitness for any of the tactics in the two years studied. Our empirical study demonstrates that the differentiation between conditional and mixed strategies is not absolute, which gives empirical support to theoretical work criticising the categorization provided by Gross (Shuster & Wade 2003; Tomkins & Hazel 2007; Taborsky *et al.* 2008). In many other species, environmental conditions might fluctuate temporally and spatially so that the usually sub-optimal tactic yields similar fitness to the dominant tactic, or that only a single tactic prevails (see for example Müller *et al.* 2006). We therefore suggest replacing the terms mixed and conditional strategy by the term single strategy for studies on ARTs (Box 3). The term single strategy indicates that all individuals follow a single set of decision rules determining their tactic, while the environmental conditions determine relative fitness outcomes (Schradin & Lindholm 2011).

Sex-biased dispersal and indication for alternative dispersal tactics

In striped mice, males are the dispersing sex while females are mainly philopatric, as demonstrated by the fine-scale genetic structure (Solmsen *et al.* 2011). A population genetic study identified male migrants between sub-populations and provided an indication for alternative male dispersal tactics (Solmsen *et al.* 2011): (1) highly competitive males (i.e. heavy males) have short dispersal distances, becoming the breeding males of neighbouring groups while minimizing costs of dispersal; (2) less competitive males have to disperse greater distances and become roamers in their natal sub-population; and (3) males of even lower competitive ability might not be able to disperse into areas occupied by other territorial striped mice. These males make the best of a bad job, leaving their sub-population to avoid territorial encounters, dispersing several kilometres across areas unoccupied by striped mice, until they reach another sub-population. While males migrating across unoccupied habitats can potentially create gene flow between sub-populations, this tactic is likely to be very costly in terms of energy expenditure, increased predation risk, and the peril of not finding another sub-population.

Endocrine mechanisms of alternative male reproductive tactics

Social flexibility as a response to changing environmental conditions needs physiological mechanisms that enable individuals to change their reproductive and social behaviour. The relative plasticity hypothesis predicts that changes and differences in sex steroid hormone levels regulate the expression of alternative reproductive tactic (Moore *et al.* 1998). In species with alternative male reproductive tactics, the highest androgen levels have usually been reported in dominant males (Oliveira *et al.* 2008). However, in sociable species, amicable behaviours may conflict with high testosterone levels. In the striped mouse, territorial breeders show highly amicable behaviours towards other group members (Schradin & Pillay 2004; Schubert *et al.* 2009), and have lower testosterone levels than solitary roamers, which might reflect a trade-off between low testosterone amicable behaviour and high testosterone dominance behaviour (Schradin *et al.* 2009b). Territorial breeders are heavier than roamers, and territory holding potential may be related more to body mass than to testosterone levels. The high testosterone levels of roamers, on the other hand, might promote risky behaviour, such as invading territories defended by territorial males. Philopatric males have the highest corticosterone but lowest testosterone levels, indicating that they are sexually suppressed and potentially stressed by being forced (by ecological constraints) to remain philopatric instead of breeding independently. Prolactin, a hormone associated with paternal care (Schradin & Anzenberger 1999), was highest in territorial breeders (Schradin 2008a), which show high levels of paternal care (Schradin & Pillay 2003). Interestingly, the differences in hormone levels between tactics disappeared during the non-breeding season (Schradin 2008a, b), suggesting that they were rather due to differences in reproductive

behaviour than in dominance rank. Important differences were also found in resting metabolic rate (RMR), which was higher in the two group-living tactics than in the solitary roamers. Energy savings due to huddling in a social group by males adopting social tactics might enable higher energy expenditure during the day and a higher RMR, which could lead to a better reaction potential, enabling for example a faster response towards predators (Schradin *et al.* 2009b).

The endocrine differences found in the field were also demonstrated in the laboratory (Schradin *et al.* 2009c). When brothers were separated at an age of 21 days (weaning is at day 16), with one brother remaining in the family (representing a philopatric male) and the other male housed singly (representing a solitary roamer), the singly housed male reached puberty earlier (became scrotal with descended testes) than his family living brother. After both males were sexually mature, they differed hormonally in the same way as do philopatrics and roamers in the field, with the family housed males exhibiting higher corticosterone but lower testosterone levels. Furthermore, family-housed males had smaller testes and lower sperm counts. This only occurred in families were the father was present, but not when the father was removed, indicating sexual suppression by the breeding male. This was supported by field data from 170 individuals: males were found to became sexually mature at a younger age when no breeding male was present in their group and when food was abundant (Schradin *et al.* 2009c). Onset of puberty in male striped mice is therefore flexible, with environmental cues providing the relevant information on resource availability and opportunities for reproduction (Schradin *et al.* 2009c).

Extra-group paternity and female choice

Extra-group paternity is common in striped mice, and it is more common for pups from young compared to old females (87% *vs.* 20%; compare Fig. 4B with C), indicating inbreeding avoidance by young females. While territorial breeding males have high reproductive success, they nevertheless loose more than 30% of within-group paternity to other males, mainly neighbouring territorial breeders (Schradin *et al.* 2010b; Fig. 4A). In neutral arena experiments, territorial breeders were found to be especially aggressive towards their direct neighbours, the so-called 'nasty neighbour phenomenon' (Schradin *et al.* 2010b). This is in contrast to the 'dear enemy phenomenon', where neighbours show reduced levels of aggression towards each other, while being more aggressive towards strangers (Temeles 1994). Aggression in male striped mice seems to be a response to the high risk of extra-pair copulations, and we predicted that with increasing rate of extra-pair fertilizations by neighbours, animals will rather show the 'nasty neighbour' instead of the 'dear enemy phenomenon' (Schradin *et al.* 2010b).

Synthesis: why is social flexibility important?

In species in which both males and females have alternative reproductive tactics based on a single strategy, the entire social system can switch, a phenomenon we refer to as social flexibility. Thus, when studying social flexibility, the focus is consistently on the individual and how its decisions affect the social system of a population. Social flexibility is one of the hallmarks of humans, and its understanding will provide insights into our own behaviour. We focused on social flexibility in African striped mice to study aspects of group living by comparing solitary and social individuals of the same population, without confounding phylogenetic differences. By using molecular tools and studying several generations, we demonstrated important differences in fitness outcomes, mate choice and dispersal tactics, depending on prevailing environmental conditions and individual responses to the environment, helping us to understand many different aspects of social behaviour (Box 2) and developing new concepts (Boxes 1 and 3).

Social flexibility is in itself an interesting and important phenomenon that needs scientific explanation. When does social flexibility evolve and why is it not present in many more species? At the moment we can merely speculate about the evolutionary forces. Social flexibility might be a coping mechanism for individuals to survive and reproduce in unpredictably fluctuating environments (Randall *et al.* 2005). Cooperative breeding in birds, an alternative to breeding in pairs, has been found to occur more often in unstable environments (Jetz & Rubenstein 2011). We predict that unpredictably recurring environmental changes lead to selection for genotypes that enable high phenotypic flexibility (a broad reaction norm). Regarding social behaviour, individuals must have genotypes enabling them to tolerate other individuals for long periods (longer than mating) and the motivation to seek their company (leading to group-living), and a switch to a motivational state to avoid other individuals (leading to solitary- living). This might be especially important in short living species such as the striped mouse, where one generation lives for 1 year and has to maximize its lifetime reproductive success under the prevailing environmental conditions, which might differ significantly from the conditions experienced by previous and succeeding generations. Thus, if environmental conditions

change faster than genetic adaptations can occur, social flexibility allows for an immediate response. While social flexibility enables adaptation to a certain range of a fluctuating environment, anthropogenic (climate) change might induce conditions to which individuals can no longer adapt behaviourally and physiologically. In a period of accelerated climate change it is important to know the limits of social flexibility to predict the range of conditions to which individuals can respond adaptively.

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References

Bergmüller R, Heg D, Taborsky M (2005) Helpers in a cooperatively breeding cichlid stay and pay or disperse and breed, depending on ecological constraints. *Proceedings of the Royal Society of London B*, **272**, 325–331.

Berry RJ, Tattersall FH, Hurst J (2008) Genus *Mus*. In: *Mammals of the British Isles Handbook*, 4th edn (eds Harris S, Yalden DW), pp. 141–149. The Mammal Society, Southampton, UK.

Bothma J du P, Walker C (1999) *Larger Carnivores of the African Savannas*. pp. 277. J.L. van Schaik, Pretoria.

Canals M, Rosenmann M, Bozinovic F (1989) Energetics and geometry of huddling in small mammals. *Journal of theoretical Biology*, **141**, 181–189.

Carter CS, Getz LL (1993) Monogamy and the prairie vole. *Scientific American*, **6**, 70–76.

Clutton-Brock TH, Sheldon BC (2010) Individuals and populations: the role of long-term, individual-based studies of animals in ecology and evolutionary biology. *Trends in Ecology & Evolution*, **25**, 562–573.

Clutton-Brock TH (2005) Cooperative breeding in mammals. In: *Cooperation in Primates and Human* (eds Kappeler PM, van Schaik CP). pp. 173–190, Springer, Heidelberg.

Cushing BS, Razzoli M, Murphy AZ, *et al.* (2004) Intraspesific variation in estrogen receptor alpha and the expression of male sociosexual behavior in two populations of prairie voles. *Brain Research*, **1016**, 247–254.

Davies NB (1992) *Dunnock Behaviour and Social Evolution*. Oxford University Press, Oxford.

Dawkins R (1980) In: *Good strategy or evolutionary stable strategy? In: Sociobiology: Beyond Nature/Nurture* (eds Barlow GW, Silverberg J). pp. 331–367, Westview, Boulder, CO.

Dobson FS (1982) Competition for mates and predominant juvenile male dispersal in mammals. *Animal Behaviour*, **30**, 1183–1192.

Dunbar RIM (2002) Modelling primate behavioral ecology. *International Journal of Primatology*, **23**, 785–819.

Eggert A.-K (1992) Alternative male mate-finding tactics in burying beetles. *Behavioral Ecology*, **3**, 243–254.

Eggert A-K, Müller JK (2000) Timing of oviposition and reproductive skew in cobreeding female burying beetles (*Nicrophorus vespilloides*). *Behavioral Ecology*, **11**, 357–366.

Elwood RW, Kennedy HF (1991) Selectivity in paternal and infanticidal responses by male mice effects of relatedness location and previous sexual partners. *Behavioural and Neural Biology*, **56**, 129–147.

Emlen ST (1997) Predicting family dynamics in social vertebrates. In: *Behavioural Ecology* (eds Krebs JR, Davies NB), pp. 228–253. Blackwell Science, Oxford.

Faulkes CG, Bennett NC (2001) Family values: group dynamics and social control of reproduction in African mole-rats. *Trends in Ecology and Evolution*, **16**, 184–190.

Friedlingstein P (2008) A steep road to climate stabilization. *Nature*, **451**, 297–298.

Gross MR (1996) Alternative reproductive strategies and tactics: diversity within the sexes. *Trends in Ecology and Evolution*, **11**, 92–98.

Heimann M, Kasermann HP, Pfister R, Roth DR, Burki K (2009) Blood collection from the sublingual vein in mice and hamsters: a suitable alternative to retrobulbar technique that provides large volumes and minimizes tissue damage. *Laboratory Animals*, **43**, 255–260. doi: 10.1258/la.2008.007073.

Jackson TP (1999) The social organisation and breeding system of Brants' whistling rat (*Parotomys brantsii*). *Journal of Zoology*, **247**, 323–331.

Jetz W, Rubenstein DR (2011) Environmental uncertainty and the global biogeography of cooperative breeding in birds. *Current Biology*, **21**, 72–78.

Kappeler PM, van Schaik CP (2002) Evolution of primate social systems. *International Journal of Primatology*, **23**, 707–740.

Koenig WD, Mumme RL, Stanback MT, Pitelka FA (1995) Patterns and consequences of egg destruction among joint-nesting acorn woodpeckers. *Animal Behaviour*, **50**, 607–621.

Koenig WD, Pitelka FA, Carmen WJ, Mumme RL, Stanback MT (1992) The evolution of delayed dispersal in cooperative breeders. *Quarterly Review of Biology*, **67**, 111–150.

Kokko H. (2003) Are reproductive skew models evolutionary stable? *Proceedings Royal Society of London B*, **270**, 265–270.

Komdeur J (1992) Importance of habitat saturation and territory quality for evolution of cooperative breeding in the Seychelles warbler. *Nature*, **358**, 493–495.

Kraaijeveld K, Dickinson JL (2001) Family-based winter territoriality in western bluebirds, *Sialia mexicana*: the structure and dynamics of winter groups. *Animal Behaviour*, **61**, 109–117.

Krause J, Ruxton GD (2002) *Living in Groups*. Oxford University Press, Oxford.

Krebs JR, Davies NB (1993) *An Introduction to Behavioural Ecology*, 3rd edn. Blackwell Science Ltd, Oxford.

Lank DB, Smith CM, Hanotte O, Burke T, Cooke F (1995) Genetic polymorphism for alternative mating behaviour in lekking male ruff *Philomachus pugnax*. *Nature*, **378**, 59–62.

Latham N, Mason G (2004) From house mouse to mouse house: the behavioural biology of free-living *Mus musculus*

and its implications in the laboratory. *Applied Animal Behaviour Science*, **86**, 261–289.
Lott DF (1984) Intraspecific variation in the social systems of wild vertebrates. *Behaviour*, **88**, 266–325.
Lott DF (1991) *Intraspecific Variation in the Social Systems of Wild Vertebrates*. Cambridge University Press, New York.
Lucia KE, Keane B, Hayes LD, Lin YK, Schaefer RL, Solomon NG (2008) Philopatry in prairie voles: an evaluation of the habitat saturation hypothesis. *Behavioral Ecology*, **19**, 774–783.
Mabry KE, Streatfeild CA, Keane B, Solomon NG (2011) *avpr1a* length polymorphism is not associated with either social or genetic monogamy in free-living prairie voles. *Animal Behaviour*, **81**, 11–18.
Madison DM, FitzGerald RW, McShea WJ (1984) Dynamics of social nesting in overwintering meadow voles (*Microtus pennsylvanicus*): possible consequences for population cycling. *Behavioral Ecology and Sociobiology*, **15**, 9–17.
Moore MC, Hews DK, Knapp R (1998) Hormonal control and evolution of alternative male phenotypes: generalizations of models for sexual differentiation. *American Zoologist*, **38**, 133–151.
Müller JF, Braunisch V, Hwang W, Eggert A-K (2006) Alternative tactics and individual reproductive success in natural associations of the burying beetle, *Nicrophorus vespilloides*. *Behavioral Ecology*, **18**, 196–203.
Oliveira RF, Canario AVM, Ros AFH (2008) Hormones and alternative reproductive tactics in vertebrates. In: *Alternative Reproductive Tactics* (eds Oliveira RF, Taborsky M, Brockmann HJ), pp. 132–174. Cambridge University Press, Cambridge.
Ophir AG, Campbell P, Hanna K, Phelps SM (2008a) Field tests of cis-regulatory variation at the prairie vole avpr1a locus: Association with V1aR abundance but not sexual or social fidelity. *Hormones and Behavior*, **54**, 694–702.
Ophir AG, Phelps SM, Sorin AB, Wolff JO (2008b) Social but not genetic monogamy is associated with greater breeding success in prairie voles. *Animal Behaviour*, **75**, 1143–1154.
Pillay N. (2000) Fostering in the African striped mouse: implications for kin recognition and dominance. *Acta Theriologica*, **45**, 193–200.
Pruett-Jones SG, Lewis MJ (1990) Sex ratio and habitat limitation promote delayed dispersal in superb fairy-wrens. *Nature*, **348**, 541–542.
Purcell J, Aviles L (2007) Smaller colonies and more solitary living mark higher elevation populations of a social spider. *Journal of Animal Ecology*, **76**, 590–597.
Randall JA, Rogovin K, Parker PG, Eimes JA (2005) Flexible social structure of a desert rodent, *Rhombomys opimus*: philopatry, kinship, and ecological contraints. *Behavioral Ecology*, **16**, 961–973.
Ragsdale JE (1999) Reproductive skew theory extended: the effect of resource inheritance on social organisation. *Evolutionary Ecology Research*, **1**, 859–874.
Scantlebury M, Bennett NC, Speakman JR, Pillay N, Schradin C (2006) Huddling in groups leads to daily energy savings in free-living African four-striped grass mice, *Rhabdomys pumilio*. *Functional Ecology*, **20**, 166–173.
Schradin C (2004) Territorial defense in a group living solitary forager: who, where, against whom? *Behavioral Ecology and Sociobiology*, **55**, 439–446.
Schradin C (2005) When to live alone and when to live in groups: ecological determinants of sociality in the African striped mouse (*Rhabdomys pumilio*, Sparrman, 1784). *Belgian Journal of Zoology*, **135**(suppl.), 77–82.
Schradin C (2006) Whole day follows of the striped mouse. *Journal of Ethology*, **24**, 37–43.
Schradin C (2007) Information transfer about food locations is not a benefit of group living in the solitary foraging striped mouse (*Rhabdomys pumilio*). *Journal of Ethology*, **25**, 83–86.
Schradin C (2008a) Differences in prolactin levels between three alternative male reproductive tactics in striped mice (*Rhabdomys pumilio*). *Proceedings of the Royal Society of London B*, **275**, 1047–1052.
Schradin C (2008b) Seasonal changes in testosterone and corticosterone levels in four social categories of a desert dwelling sociable rodent. *Hormones and Behavior*, **53**, 573–579.
Schradin C, Anzenberger G (1999) Prolactin, the hormone of paternity. *News in Physiological Sciences*, **14**, 223–231.
Schradin C, Kinahan AA, Pillay N (2009a) Cooperative breeding in groups of synchroneously mating females and evolution of large testes to avoid sperm depletion in African striped mice. *Biology of Reproduction*, **81**, 111–117.
Schradin C, König B, Pillay N (2010a) Reproductive competition favours solitary living while ecological constraints impose group-living in African striped mice. *Journal of Animal Ecology*, **79**, 515–521.
Schradin C, Krackow S, Schubert M, Keller C., Schradin B., Pillay N (2007) Regulation of activity in desert-living striped mice: The importance of basking. *Ethology*, **113**, 606–614.
Schradin C, Lindholm AK (2011) Relative fitness of alternative male reproductive tactics in a mammal varies between years. *Journal of Animal Ecology*, **80**, 908–917. Doi: 10.1111/j.1365-2656.2011.01831.x
Schradin C, Pillay N (2003) Paternal care in the social and diurnal striped mouse (*Rhabdomys pumilio*): laboratory and field evidence. *Journal of Comparative Psychology*, **117**, 317–324.
Schradin C, Pillay N (2004) The striped mouse (*Rhabdomys pumilio*) from the succulent karoo of South Africa: A territorial group living solitary forager with communal breeding and helpers at the nest. *Journal of Comparative Psychology*, **118**, 37–47.
Schradin C, Pillay N (2005) The influence of the father on offspring development in the striped mouse. *Behavioral Ecology*, **16**, 450–455.
Schradin C, Scantlebury M, Pillay N, König B (2009b) Testosterone levels in dominant sociable males are lower than in solitary roamers: Physiological differences between three male reproductive tactics in a sociably flexible mammal. *American Naturalist*, **173**, 376–388.
Schradin C, Schneider C, Lindholm AK (2010b) The nasty neighbour in the striped mouse (*Rhabdomys pumilio*) steals paternity and elicits aggression. *Frontiers in Zoology*, **7**, 19.
Schradin C, Schneider C, Yuen CH (2009c) Age at puberty in male African striped mice: the impact of food, population density and the presence of the father. *Functional Ecology*, **23**, 1004–1013.
Schradin C, Schubert M, Pillay N (2006) Winter huddling groups in the striped mouse. *Canadian Journal of Zoology*, **84**, 693–698.
Schubert M, Pillay N, Schradin C (2009) Parental and allo-parental care in a polygynous mammal. *Journal of Mammalogy*, **90**, 724–731.

Shuster SM, Sassaman C (1997) Genetic interaction between male mating strategy and sex ratio in a marine isopod. *Nature*, **388**, 373–376.

Shuster SM, Wade MJ (2003) *Mating Systems and Strategies*. Princeton University Press, Princeton.

Solmsen N, Johannesen J, Schradin C (2011) Highly asymmetric fine-scale genetic structure between sexes of African striped mice and indication for condition dependent alternative male dispersal tactics. *Molecular Ecology*, **20**, 1624–1634.

Solomon NG, Richmond AR, Harding PA, *et al.* (2009) Polymorphism at the *avpr1a* locus in male prairie voles correlated with genetic but not social monogamy in field populations. *Molecular Ecology*, **18**, 4680–4695.

Taborsky M (1984) Broodcare helpers in the cichlid fish *Lamprologus brichardi:* their costs and benefits. *Animal Behaviour*, **32**, 1236–1252.

Taborsky M, Oliveira RF, Brockmann HJ (2008). The evolution of alternative reproductive tactics: concepts and questions. In: *Alternative Reproductive Tactics* (eds Oliveira RF, Taborsky M, Brockmann HJ), pp. 1–22. Cambridge University Press, Cambridge.

Temeles EJ (1994) The role of neighbours in territorial systems: when are they 'dear enemies'? *Animal Behaviour*, **47**, 339–350.

Tomkins JL, Hazel W (2007) The status of the conditional evolutionary stable strategy. *Trends in Ecology and Evolution*, **22**, 522–528.

Wcislo WT, Danforth BN (1997) Secondarily solitary: the evolutionary loss of social behavior. *Trends in Ecology & Evolution*, **12**, 468–474.

Webster AB, Brooks RJ (1981) Social behavior of *Microtus pennsylvanicus* in relation to seasonal changes in demography. *Journal of Mammalogy*, **62**, 738–751.

Wingfield JC (2003) Control of behavioural strategies for capricious environments. *Animal Behaviour*, **66**, 807–816.

Wingfield JC, Sapolsky RM (2003) Reproduction and Resistance to stress: when and how. *Journal of Neuroendocrinology*, **15**, 711–724.

Young L, Wang Z (2004) The neurobiology of pair bonding. *Nature Neuroscience*, **7**, 1048–1054.

Young L (2009) Love: Neuroscience reveals all. *Nature*, **457**, 148.

Young AJ, Spong G, Clutton-Brock T (2007) Subordinate male meerkats prospect for extra-group paternity: alternative reproductive tactics in a cooperative mammal. *Proceedings of the Royal Society of London B*, **274**, 1603–1609.

Chapter 5

Invasion success and genetic diversity of introduced populations of guppies *Poecilia reticulata* in Australia

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Invasion success and genetic diversity of introduced populations of guppies *Poecilia reticulata* in Australia

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Abstract

High genetic diversity is thought to characterize successful invasive species, as the potential to adapt to new environments is enhanced and inbreeding is reduced. In the last century, guppies, *Poecilia reticulata*, repeatedly invaded streams in Australia and elsewhere. Quantitative genetic studies of one Australian guppy population have demonstrated high additive genetic variation for autosomal and Y-linked morphological traits. The combination of colonization success, high heritability of morphological traits, and the possibility of multiple introductions to Australia raised the prediction that neutral genetic diversity is high in introduced populations of guppies. In this study we examine genetic diversity at nine microsatellite and one mitochondrial locus for seven Australian populations. We used mtDNA haplotypes from the natural range of guppies and from domesticated varieties to identify source populations. There were a minimum of two introductions, but there was no haplotype diversity within Australian populations, suggesting a founder effect. This was supported by microsatellite markers, as allelic diversity and heterozygosity were severely reduced compared to one wild source population, and evidence of recent bottlenecks was found. Between Australian populations little differentiation of microsatellite allele frequencies was detected, suggesting that population admixture has occurred historically, perhaps due to male-biased gene flow followed by bottlenecks. Thus success of invasion of Australia and high additive genetic variance in Australian guppies are not associated with high levels of diversity at molecular loci. This finding is consistent with the release of additive genetic variation by dominance and epistasis following inbreeding, and with disruptive and negative frequency-dependent selection on fitness traits.

Keywords: additive genetic variation, bottleneck, introduced species, invasion success, neutral genetic diversity, *Poecilia reticulata*

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Introduction

Species that invade or that are introduced into new environments provide opportunities to study evolutionary change and diversification, particularly the consequences of random genetic drift and natural selection (Endler 1986; Lee 2002). If populations in new environments are founded by a small number of individuals, or if they go through bottlenecks after reintroduction, allelic diversity will be reduced relative to the source population (Nei *et al*. 1975). Both drift and mating between related individuals will result in an increased probability that two alleles in a given individual at a given locus are identical by descent. This probability is the coefficient of inbreeding, *f*. A rise in the mean coefficient of inbreeding within a population is expected to lead to an erosion of additive genetic variance at the rate of $(1 - f)\, V_A$ (Falconer & Mackay 1989). Such a loss of additive genetic variance will slow evolutionary responses to selection (Falconer & Mackay 1989), potentially

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constraining introduced populations from adapting to the new environment and thus invading it successfully.

Three alternative processes may free invading populations from the constraints of reduced additive genetic variation. First, in small populations, dominance and epistasis may result in an increase in additive genetic variance, particularly under inbreeding (Goodnight 1988; Willis & Orr 1993). Second, negative frequency dependence may maintain high levels of additive genetic variation (Barton & Turelli 1989; Falconer & Mackay 1989). Last, populations in new environments may have been established via multiple introductions or via admixture or hybridization of different source populations, resulting in enhanced allelic diversity, additive genetic variance and heritability relative to source populations that will increase evolutionary response to selection (Ellstrand & Schierenbeck 2000; Grant & Grant 2000; Kolbe *et al*. 2004).

Guppies, which are small live-bearing fish, offer a particularly interesting opportunity to study invasion success as there is a wealth of information on their biology (Houde 1997). Moreover, through accidental or deliberate release, guppies have successfully colonized at least 32 countries in the Americas, Europe, Asia, Australasia, and Africa (Froese & Pauly 2000; FAO 2004). They are native to South America from Venezuela to Brazil and nearby islands (Rosen & Bailey 1963; Welcomme 1988).

In Australia, multiple introductions of guppies are likely to have occurred. They were probably first brought to Australia around 1910. At this time guppies were sent through the Colonial Office to various tropical colonies for mosquito control (Vipan 1910). By 1912 guppies were already present in Australia, as the efficacy of the guppy in mosquito larvae control was tested in Brisbane in Queensland (Cooling 1912) and later in Adelaide in South Australia (Borthwick 1923). Several city councils, including that of Brisbane (Abell 1913; Borthwick 1923), likely played a role in the dissemination of guppies by encouraging the addition of mosquito-eating fish to stagnant water bodies. In 1929 the guppy was listed among mosquito-eating fish introduced to Australia (Hamlyn-Harris 1929). During World War II, it is believed that soldiers spread guppies and *Gambusia* in northern Queensland for biocontrol (J. Johnson, personal communication), a period in which there was an epidemic of malaria in Cairns (Patrick 1987). Today, guppies primarily occur in Queensland near sugarcane fields, where they may also have been used for mosquito control (McKay 1987), and in coastal drainages near urban areas (Allen *et al*. 2002).

Guppies are also popular ornamental fish in Australia, and introductions may have resulted from escapes or releases from aquaria or outdoor breeding ponds (McKay 1984). The most important source of ornamental guppies in the Australian pet trade until very recently has been Singapore (Glenn Briggs, personal communication). Guppies were introduced to Singapore in the 1900s (FAO 2004) and were found in feral populations in streams before 1940 (Herre 1940). From 1972–1977, Singapore exported more than 43 million tropical fish to Australia (UNCTAD/GATT 1979). From 1992 to 1994, Singapore was the top exporter of tropical fish in the world, and its main export was the guppy (Cheong 1996). If introductions resulted from releases of ornamental fish, then Singapore would be the most likely immediate source. Ornamental or 'fancy' guppies would have undergone artificial selection and inbreeding during domestication, and would be expected to have a different genetic architecture than either wild populations or populations resulting from introductions for biocontrol.

High levels of additive genetic variance have been found in laboratory studies of two introduced populations of guppies. In an introduced Australian population (Alligator in this study), male body size and colour traits showed high heritabilities (h^2) and coefficients of additive genetic variation (CV_A). Of 13 traits measured, overall h^2 estimates of seven traits were above 0.50, while CV_A estimates from six were above 20% (e.g. for body area $h^2 = 0.87$ and $CV_A = 11.8$ and for orange area $h^2 = 0.96$ and $CV_A = 67.3$; Brooks & Endler 2001a). In the same population, female mate preference functions were negligibly heritable (Brooks & Endler 2001b). An introduced South African population showed responses to artificial selection consistent with high heritability for both male colour and female preferences (Brooks & Couldridge 1999), as has been found in native populations (Houde 1994). For introduced guppies, high heritability of male colour traits is remarkable as much of the variation for these traits is Y-linked (Brooks & Endler 2001). Like mitochondrial loci, Y-linked loci have only one quarter of the effective population size of autosomal loci (Halliburton 2004). In guppies, variance in reproductive success of males (Becher & Magurran 2004) will further reduce the effective population size of Y chromosomes. Genetic bottlenecks should therefore have a particularly strong impact on variation in Y-linked traits in guppies.

Introduced populations, such as those in Australia and South Africa, may have high levels of additive genetic variance as a result of population admixture through multiple introductions, through the release of additive genetic variance by dominance and epistatic interactions among alleles, or through disruptive and negative frequency-dependent selection. If the first scenario pertains, neutral genetic markers will show similarly high levels of variation, whereas the latter two scenarios are consistent with small amounts of genetic variation typical of single introductions. In this study we examine genetic diversity in seven introduced guppy populations in northern Queensland, Australia, using nine autosomal microsatellite loci and one mtDNA locus, and use these to test the prediction of high

genetic diversity due to admixture of populations during multiple introductions. As mtDNA is especially suited to tracing founder events (Moritz *et al*. 1987), we use mtDNA haplotype diversity to estimate how many source populations there were, and their geographical origin. We also explore the hypothesis that Australian feral guppies descend from ornamental fish bred in Singapore.

Materials and methods

Sampling

Adult male and female guppies were captured from 5–12 April 2002 at seven sites in north Queensland, Australia (Fig. 1; Table 1). Guppies were air transported to Sydney and populations were separately housed in large tanks in a greenhouse at the University of New South Wales for use in several studies. Tissue samples were obtained from tailfin clips of live animals, or from muscle tissue of animals that were euthanized or died naturally. Thirty-nine guppies and one *Poecilia parae* [a sister taxon (Breden *et al*. 1999)] were also sampled from Patientia near Georgetown, Guyana, South America, in February 2002. Samples were stored in 20% DMSO salt solution (Amos & Hoelzel 1991) before DNA extraction. DNA was isolated from all samples by salt precipitation using Puregene Tissue Kit (Gentra) according to manufacturer's instructions.

Singapore ornamental guppies of the inbred varieties Tuxedo (see Khoo *et al*. 1999), Greensnake (Phang *et al*. 1989) and Red Tail (see Fernando & Phang 1990) were obtained from Swee Hing & Brothers Aquarium Company, Singapore, in 1995 and kept as stocks in the Genetics Lab, Department of Biological Sciences, National University of Singapore, until 2002. One individual from each of these three stocks were sampled in 1996 with an additional individual from a laboratory stock of feral guppies collected from Nee Soon, Singapore. These four individuals were analysed in 1996. After 1995–1996, a disease outbreak in Singapore led to wide-scale replacement of local guppy stocks with those from other countries that farm guppies. In 2001 laboratory stocks were eliminated and genetic samples destroyed. We could therefore not sample additional individuals nor evaluate the effect of selective breeding on genetic diversity.

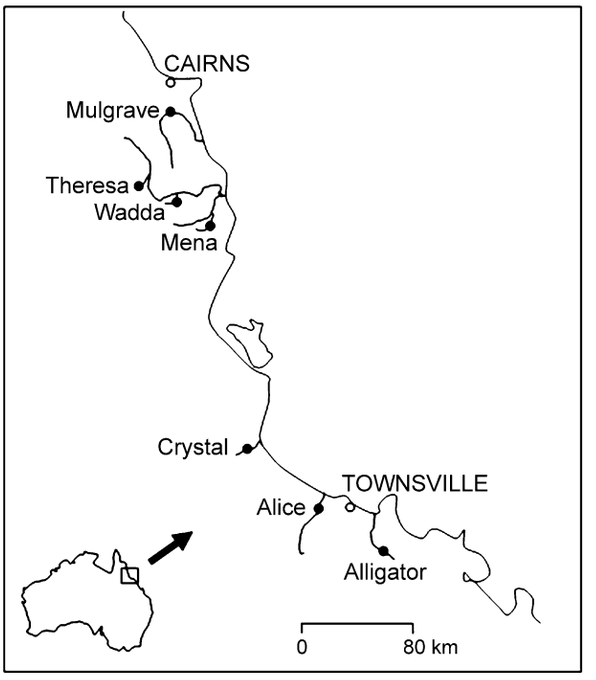

Fig. 1 Sampling locations in northern Queensland.

Measuring genetic diversity: mitochondrial DNA sequencing

Four hundred ninety-eight base pairs of the mitochondrial control region were sequenced for Queensland guppies (Table 1), two Guyanese guppies, and for *Poecilia parae*, the outgroup. Primers Lpro and 13R were used for initial amplification and sequencing (Ptacek & Breden 1998). Polymerase chain reaction (PCR) products were cleaned using the QIAquick PCR purification kit (QIAGEN). Sequencing products were ethanol precipitated and sequenced on an ABI 377 or 3730 DNA sequencer. Haplotypes were deposited in GenBank under Accession nos DQ097186–90.

Table 1 Sampling locations from south to north, relative population sizes, capture effort in person-hours, and sample sizes for mtDNA sequencing and microsatellite genotyping

Sample site (creek or river)	Location	Number captured	Capture effort	Number sequenced	Number genotyped
Alligator	19.45°S, 146.97°E	514	4.5	6	56
Alice	19.32°S, 146.60°E	6	3	6	6
Crystal	18.38°S, 146.33°E	163	8	6	52
Mena	17.65°S, 145.97°E	160	9	6	49
Wadda	17.60°S, 145.83°E	138	1	6	58
Theresa	17.50°S, 145.62°E	94	8	6	55
Mulgrave	17.12°S, 145.45°E	100	8	5	38

Identification of source populations

To determine the likely population origin of Queensland feral guppies, we compared their haplotypes to those of 128 wild guppies from the native range (see Table 2 for details) from Taylor & Breden (2000), Alexander & Breden (2004, unpublished), and this study, and three domesticated and one wild variety of Singapore guppies. Sequences from Singapore guppies were amplified using primers L15926 (Kocher *et al*. 1989) and H16498 (Shields & Kocher 1991), giving 490 bp fragments (GenBank Accession nos DQ097191-94). Sequences from the control region of native guppies and the outgroup were aligned to those of Australian guppies using CLUSTAL_X and trimmed to a uniform length, giving a 510-bp segment including gaps. The Singapore sequences overlapped this trimmed segment by 386 bp.

Phylogenetic analysis was performed using maximum likelihood, which performs well with missing data (Dunn *et al*. 2003). We first reduced the data set to a total of 66 haplotypes by excluding duplicate haplotypes from the same sampling location, and then used the likelihood-ratio test of MODELTEST version 3.5 (Posada & Crandall 1998) to select the best-fit model of nucleotide substitution for use in tree construction. We then estimated the maximum-likelihood tree using Felsenstein & Churchill's (1996) algorithm implemented in PHYLIP and calculated bootstrap support based on a majority-rule consensus of maximum-likelihood trees from 1000 bootstraps. As this algorithm treats indels as unknown nucleotides, thereby losing information, we also calculated bootstrap support using 5000 maximum-parsimony replicates in PAUP 4.10b treating gaps as a fifth state.

Table 2 mtDNA sequences used in phylogeographic analyses and the frequency of Queensland haplotypes 1 and 2 and Singapore haplotype S out of *N* animals sequenced at each location

				Freq. haplotype			
Region	Sampling area	Tributary of	*N*	1	2	S	GenBank Accession nos
Suriname	Lelydorp	Surinam River	1				AF228605
North Guyana	Patentia	Demerera River	2	1.0			DQ097187–88
North Guyana	Bartica	Essequibo River	1				AF170257
East Guyana	New Amsterdam	Berbice River	5				AF228609, DQ102578, DQ102581–83
East Guyana	Springlands	Corentyne River	3				AF228608, DQ102560, DQ102563
West Venezuela	Guanare River		6				AF170255, AF228615, AY135451, AY135457, AY135466, AY135473
East Venezuela	Isla de Margarita		1				AF228610
East Venezuela	Rio San Miguel		4				AF538280, AY135454, AY135465, AY135468
East Venezuela	Cumaná	Rio Manzanares	19				AY373767–68, AY373770–73, AY373779–80, AY373787–88, AY373791, AY373796, AY373798, AY373805–07, AY373812–14
East Venezuela	Yaguaracual		3				AY373776–78
East Venezuela	Paria Peninsula		11				AY373765–66, AY373782–6, AY373795, AY373803–04, AY373808
East Venezuela	Mira Flores/ Arenas/ Cumanocoa	Rio Manzanares	10				AY373764, AY373792–93, AY373799–802, AY373809–11
West Trinidad	Arima River	Caroni	9		0.33		AF228623, AF170265–66, AY135450, AY135452, AY135460, AY135475–77
West Trinidad	Aripo River	Caroni	4		0.25		AF170268, AY135470, DQ102585–86
West Trinidad	Guanapo River	Caroni	4		0.75		AF170267, AY135449, AY135472, AY373762
East Trinidad	Turure River	Oropuche	2		0.50		AF327266, AY135469
East Trinidad	Quare River	Oropuche	7				AF170261, AF193897–98, AF529246, AF52951–53
East Trinidad	Oropuche River		12				AF170259–60, AF193899, AF529244–45, AF529247, AF529249–50, AF529255–57, AF538279
East Trinidad	Aqui River	Madamas	3				AF170262, AF529248, AF529254
East Trinidad	Rio Grande		3				AF170258, AF170269–70
North Trinidad	Paria River		6				AF193902, AF228624, AY135448, AY135453, AY135459, AY135474
North Trinidad	Marianne River		5				AF19301, AY135456, AY135462–63, AY135467
North Trinidad	Yarra River		7				AF228625, AF170263–64, AY135455, AY135461, AY135464, AY135471
Total			128			0	

Measuring genetic diversity: microsatellites

Nine polymorphic microsatellite loci were amplified from individuals of the seven Queensland populations (Table 1): TCTG and sat 4 (Taylor 1999), TTA (Taylor 1999; but primers were redesigned), D6, D15 and D21 from Seckinger *et al*. (2002), Pr39 and Pr80 from Becher *et al*. (2002) and Pr67 (Becher & Magurran 2004). Primers were labelled with fluorescent markers and PCR products run on an ABI 377 DNA Sequencer or an ABI 3730 DNA Analyser. The mean number of loci scored per individual was 8.97. There was no evidence that these loci deviated from linkage equilibrium, or from Hardy–Weinberg equilibrium within each population, tested using FSTAT version 2.9.3 (Goudet 2001) and Bonferroni adjustments to significance tests, thereby meeting assumptions of the following tests.

Testing for population admixture

We first examined population structure to determine whether the number of genetic clusters estimated from mtDNA haplotypes correspond to that of nuclear markers. STRUCTURE 2.1 uses a Bayesian model implemented by a Markov chain Monte Carlo method to estimate how many different populations (genetic clusters in Hardy–Weinberg equilibrium) are in a data set, irrespective of sampling location (Pritchard *et al*. 2000; Falush *et al*. 2003). We estimated the most likely value of K (number of populations) using the admixture model with correlated allele frequencies, as populations might be closely related to each other.

If there were no admixture of populations with different mtDNA haplotypes, then populations sharing mtDNA haplotypes should be more similar to each other in microsatellite allele frequencies than populations of a different haplotype. Using a nested AMOVA design, we partitioned the estimated variance in microsatellite allele frequencies into the proportion attributable to differences in mtDNA haplotypes, the proportion attributable to populations within haplotypes, and that due to individual variation within populations.

We then examined genetic relationships between populations on a finer scale. As the Australian populations have been established over a period of less than 100 years (see Introduction), which equates to less than 200–300 generations, changes in microsatellite allele frequencies through mutation [at rates of 10^{-3} to 10^{-4} per locus per generation (Ellegren 2000)] are likely to have a minor influence on allele frequencies compared to the effects of genetic drift upon introduction. We therefore favoured methods that are based on evolutionary models of genetic drift rather than microsatellite mutation. First, we looked for evidence of gene flow between populations by identifying individuals of migrant ancestry within the data set using STRUCTURE 2.1 (Pritchard *et al*. 2000; Falush *et al*. 2003). We subsequently tested for population ancestry using the F model from STRUCTURE 2.1 (Falush *et al*. 2003). In this model a single parameter, F, analogous to F_{ST}, is calculated for each population. F estimates the degree of divergence from a common ancestral population by drift, with populations assumed to have diverged at the same time. Differences in rate of drift correspond to differences in effective population size after divergence (Nicholson *et al*. 2002; Falush *et al*. 2003). If one population has not diverged at the same time from the hypothetical ancestral population, but is rather descended from another population in the data set, its F value decreases when its ancestor is excluded (Falush *et al*. 2003). We therefore compared F values (using 40 estimates) omitting and including a likely source population for some of the Queensland populations, assuming that each sampling location represented an independent population.

As a complementary approach to investigating population relatedness, we used microsatellite allele frequencies to estimate phylogenetic trees using two methods. First, we constructed a restricted maximum-likelihood phylogenetic tree using Felsenstein's (1981) method implemented in PHYLIP. It assumes a Brownian motion model, and therefore, that loci evolve only by drift. The second method estimated a neighbour-joining tree from Nei's genetic distance D (Nei 1972), also using PHYLIP (Felsenstein 2004). Nei's genetic distance measure is based on the infinite alleles model and is the most widely used measure for codominant data (Lowe *et al*. 2004). For both trees, allele frequencies were bootstrapped 1000 times using MICROSAT (http://hpgl.stanford.edu/projects/microsat).

Testing for bottlenecks

Introduced populations are often subject to founder effects and bottlenecks that result in low genetic diversity. We looked for evidence of recent bottlenecks using BOTTLENECK version 1.2.02 (Piry *et al*. 1999). Populations undergoing reductions of effective population size often lose rare alleles at polymorphic loci. Loss of rare alleles affects heterozygosity expected at mutation–drift equilibrium more than observed heterozygosity, leading to an excess of observed heterozygosity (Luikart & Cornuet 1998). Bottleneck tests for heterozygosity excess relative to expectations under mutation–drift equilibrium using different mutation models: the infinite allele model (IAM), one-step stepwise-mutation model (SMM), and the two-phase model (TPM). For the TPM we used a model of 90% single-step mutations and 10% multistep mutations (Garza & Williamson 2001), and a variance of 10. The Wilcoxon's signed rank test, which is more powerful than the alternatives when using a small number of loci (Piry *et al*. 1999), is the reported statistic.

Results

mtDNA haplotype diversity and sources of Queensland guppies

Two haplotypes, differing by 17 nucleotides (3.4%), were found among Queensland populations, but no nucleotide diversity was found within populations. Haplotype 1 was found exclusively in guppies from Alligator and Mena, which are nonadjacent sites (Fig. 1); while haplotype 2 was found in all guppies sequenced from Alice, Crystal, Wadda, Theresa and Mulgrave. These results are consistent with founder effects, and suggest that there were a minimum of two introductions of female guppies in Queensland, with no admixture of source populations.

Sources of Queensland guppies were identified (Table 2). Guyana is very likely to be the source of haplotype 1 guppies, as the two sequences obtained from Patentia, Guyana, are identical to haplotype 1. Haplotype 2 occurred in 7 of 18 guppies from the Caroni River drainage system, in western Trinidad. This haplotype was also found in one of two guppies from the Turure River, an admixed population, the result of a translocation of male and female guppies in 1957 from the Arima River in the Caroni drainage to the Turure River in the Oropuche River drainage (Shaw *et al*. 1992; Becher & Magurran 2000). Thus, the Caroni drainage is very likely the source of haplotype 2 guppy populations. A single haplotype (named S) was found among the four Singaporean samples that differed by 2/386 nucleotides from haplotype 2, and this haplotype was not observed in any of the Australian populations.

Phylogenetic analyses (Fig. 2) showed strong bootstrap support [from maximum likelihood (94%) and from parsimony (76%)] for a clade containing haplotype 1 and Patentia. Support for the clade of guppies sampled from the Caroni River drainage, Trinidad, and haplotypes 2 and S was low (55%), indicating that more sequence data are required to fully resolve this node.

Incorporating microsatellite data to investigate population admixture

If guppies from sample sites sharing the same mtDNA haplotype were actually interbreeding populations, then most genetic variation should be captured by two genetic clusters. Analyses using STRUCTURE 2.1 (Table 3) showed that log-likelihood ratios steadily decreased as K (the number of genetic clusters in Hardy–Weinberg equilibrium) increased from one to six, and then began to plateau at K = 6. The plateau indicates that most of the genetic variation was captured in six genetic clusters, rather than two. The six clusters correspond to the sample sites Alligator, Mena, Wadda, Theresa and Mulgrave, with Alice and Crystal together as the final cluster. Seven clusters, corresponding to the seven sample sites, were slightly more likely, and eight clusters, which split Mulgrave into two populations, plus the other six sample sites, minimized the likelihood ratio. Similar results were obtained when data were analysed using the independent allele frequencies model. We then examined how individuals were assigned to genetic clusters, using K = 6, 7 and 8. With seven clusters, the estimates of membership coefficients within sample sites appeared to be maximized, and population membership was assigned consistent with population of origin except for one female identified as of migrant descent (95% probability interval: 0.798–1.0 for K = 7), regardless of the value of K. Eight clusters split Mulgrave guppies into two groups, which did not correspond to their pool of origin. These results suggest that considering each sampling location as a separate population is reasonable, that Crystal and Alice are closely related, and that the one Theresa female consistently assigned to the Wadda genetic cluster is evidence of recent gene flow between these sites.

Table 3 Log-likelihood ratios for different K

K	Ln $P(X \mid K)$
1	−7647.4
2	−6758.1
3	−6106.6
4	−5583.0
5	−5107.2
6	−4897.1
7	−4887.4
8	−4871.0
9	−4872.0

Analysis of molecular variance (Table 4) attributed 2% of variation in microsatellite allele frequencies in Australian

Table 4 Analysis of molecular variance, nesting population variance in allele frequencies at nine microsatellite loci within the two mtDNA haplotypes

Source of variation	d.f.	SS	MS	Est. var.	% variation	P value
Among haplotypes	1	229.1	229.1	0.16	1.9	0.001
Within haplotypes among populations	5	840.1	168.0	3.89	47.5	0.001
Within haplotypes within populations	307	1317.4	4.3	4.29	48.5	0.001
Total	313	2386.6	401.4	8.34		

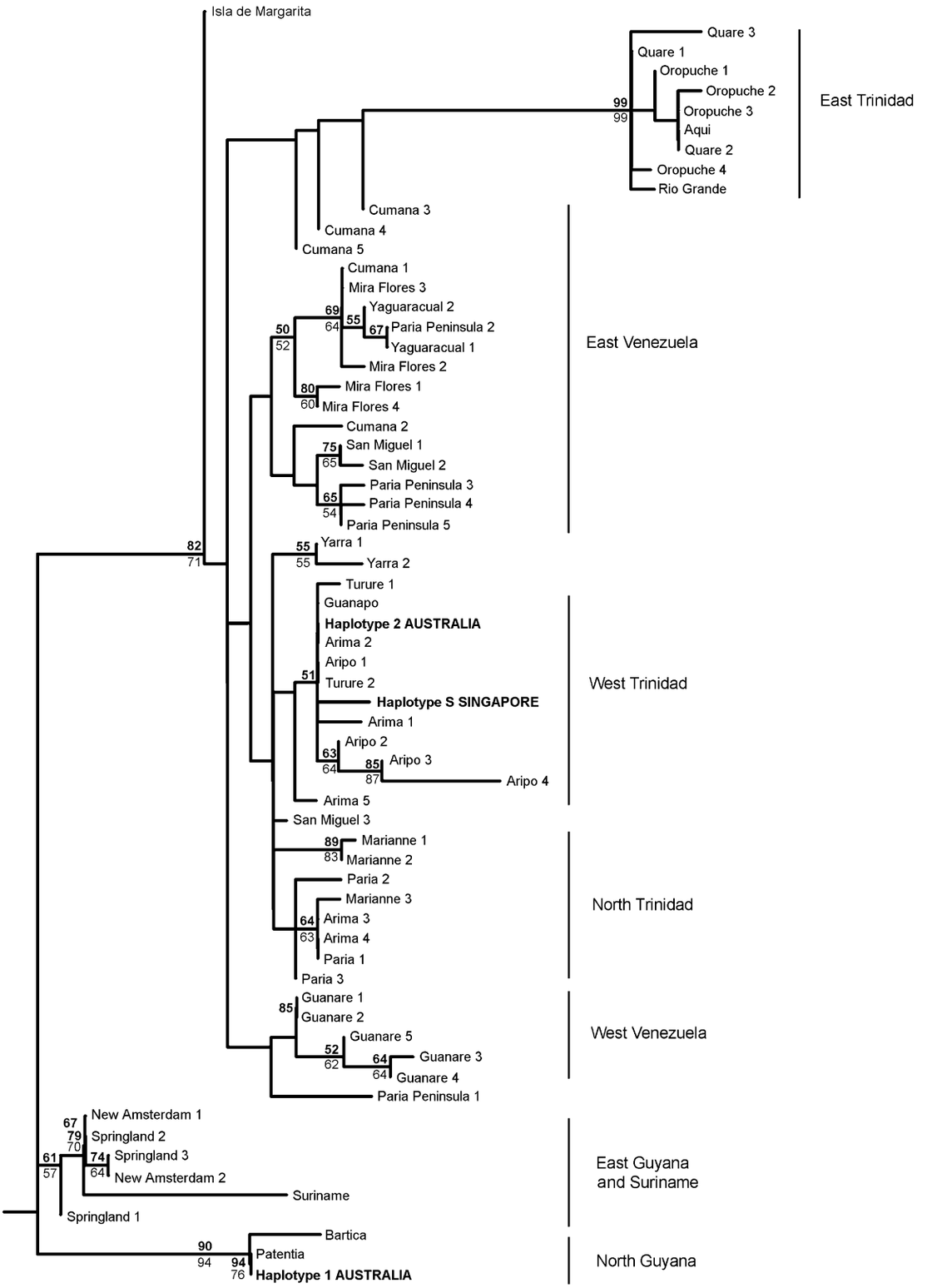

Fig. 2 Maximum-likelihood phylogenetic tree based on mtDNA control region sequence. Bootstrap values above 50% for 1000 bootstraps are indicated by the upper numbers in bold. In plain type below are bootstrap values above 50% for 5000 parsimony bootstraps. The tree was rooted using *Poecilia parae* (Breden *et al*. 1999).

populations to differences in mtDNA haplotypes, 48% of variation to population differences within haplotypes, and the remaining 49% to individual variation within populations. The small amount of microsatellite structure accounted for by mtDNA haplotype suggests that populations of the same haplotype have diverged, not only by drift, but also as a result of gene flow from populations of different haplotype.

Historical gene flow between populations of different mtDNA haplotype is further suggested by the observation that not all alleles from the haplotype 1 populations of Mena and Alligator were detected in their mtDNA source population in Guyana. All 13 of such alleles from Mena and 8 of 12 from Alligator were detected in Australian haplotype 2 populations. As an alternative explanation to gene flow, we explored whether the presence of these alleles may be due to mutations in the Mena and Alligator populations. As about 90% of microsatellite mutations appear to be single-step, and 10% multistep (Garza & Williamson 2001), we expected a similar distribution within our samples. Allele sizes did not conform to these expectations, as only 31% of the 13 alleles from Mena, and 50% of the 12 alleles from Alligator could have arisen within these populations as single-step mutations giving a change of one repeat unit. These alleles differ from nearest size classes by a mean (± 1 SD) of 4.8 ± 4.4 repeats in Mena, and 2.2 ± 1.6 repeats at Alligator. Moreover, these populations are of recent origin (see Introduction), rendering mutation an unlikely source of the majority of these alleles.

To explore population divergence by drift, we used STRUCTURE 2.1 (Falush *et al*. 2003) to estimate *F* values, which estimate the relative amount of drift that different populations have undergone from a common ancestral population (Nicholson *et al*. 2002; Falush *et al*. 2003). Estimates of *F* (Table 5) identified Wadda as the most highly differentiated of the Australian populations, while Mulgrave and Alice are the least differentiated, compared to a common ancestor. When any one Australian population was removed from the data set, only small changes in *F* were seen, suggesting that none was the ancestor of another. Adding the Guyana population to the model allowed a test of the hypothesis that it is the source of the Mena and Alligator populations. Mena showed a significant increase in *F* (along with Mulgrave and Alice), as indicated by its negative pairwise *t* statistic, while Alligator showed a nonsignificant increase (Table 5). Wadda showed the opposite trend.

Phylogenetic relationships were also inferred by tree construction using restricted maximum likelihood and neighbour joining of Nei's (1972) genetic distance measure. The trees are similar (Fig. 3a, b). In both, Alligator is the closest branch to Guyana, Alice and Crystal cluster together, while Wadda has a relatively long branch length. Bootstrap support was low overall, indicating that populations were not highly divergent in microsatellite allele frequencies.

Genetic diversity and evidence for bottlenecks

Allelic diversity and heterozygosity was lower in all Queensland populations compared to the Guyana population (Table 6), suggesting founder effects and/or bottlenecks. We found strong evidence for a recent bottleneck at both Theresa and Mulgrave (Table 7), as tests using three different models for microsatellite mutations produced similar results. At the remaining Queensland sites, with the exception of Alice, there is weak evidence for recent

Table 5 Mean values of *F*, paired sample *t*-test values and their probabilities (in bold, significant after sequential Bonferroni correction) for each population in Queensland, before and after adding Guyana to the data set

		Alligator	Alice	Crystal	Mena	Wadda	Theresa	Mulgrave	Guyana
All Queensland	Mean *F*	0.371	0.208	0.397	0.296	0.519	0.403	0.179	
	SD	0.023	0.042	0.004	0.017	0.003	0.013	0.006	
Include Guyana	Mean *F*	0.378	0.287	0.395	0.347	0.457	0.401	0.269	0.130
	SD	0.003	0.052	0.003	0.004	0.002	0.002	0.002	0.003
	Paired *t*	−2.09	−10.20	2.42	−17.99	106.52	1.17	−96.53	
	P	0.043	**0.001**	0.020	**0.001**	**0.001**	0.251	**0.001**	

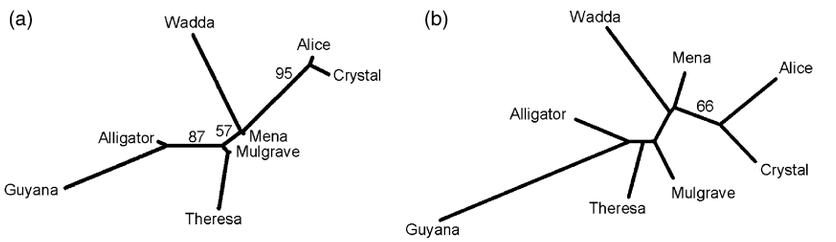

Fig. 3 Phylogenetic trees based on microsatellite allele frequencies (a) maximum-likelihood tree (b) neighbour-joining tree of Nei's genetic distance (Nei 1972). Bootstrap values above 50% for 1000 replicates are indicated.

Table 6 Number of alleles over all microsatellite loci, allelic diversity (mean number of alleles) and mean observed and expected heterozygosity

Site	Number of alleles	A	H_O	H_E
Alligator	34	3.8	0.41	0.42
Alice	30	3.3	0.63	0.53
Crystal	31	3.4	0.49	0.48
Mena	34	3.8	0.54	0.53
Wadda	21	2.3	0.36	0.34
Theresa	30	3.3	0.51	0.48
Mulgrave	42	4.7	0.61	0.64
Guyana	85	9.4	0.71	0.68

Table 7 One-tailed P values for a Wilcoxon test of heterozygote excess under three mutation models (BOTTLENECK version 1.2.02)

Site	IAM	SMM	TPM
Alligator	0.027	0.809	0.680
Alice	0.285	0.633	0.633
Crystal	0.014	0.590	0.455
Mena	0.010	0.500	0.410
Wadda	0.024	0.125	0.102
Theresa	0.004*	0.008	0.004*
Mulgrave	0.001*	0.064	0.007*
Guyana	0.150	0.787	0.752

*Significant after sequential Bonferroni correction.

bottlenecks, with significant results only before Bonferroni correction and for the least conservative model, the infinite alleles model. If the populations of Mena and Alligator are assumed to descend from the Guyanese population, and the effects of gene flow and any other processes that affect Hardy–Weinberg equilibria are ignored, then the inbreeding coefficient (f) can be calculated using the equation $H_f = H_r(1 - f)$, where H_f is heterozygosity in an inbred population and H_r is heterozygosity in a reference population (Halliburton 2004). Under these assumptions, $f = 0.38$ for Alligator, and $f = 0.22$ for Mena, indicating substantial population-level increases in the inbreeding coefficient relative to the Guyanese population.

Discussion

Genetic diversity was low in all Queensland populations: a single mtDNA haplotype was found in each population, while genetic diversity (heterozygosity and allelic diversity) at microsatellite loci was low in all Queensland populations relative to a wild population. These patterns accord with expectations of neutral genetic diversity in populations founded by a small number of individuals from the same source and/or which have passed through small bottlenecks (Nei *et al*. 1975). Furthermore, there was strong evidence that two Queensland populations have recently gone through a bottleneck.

Despite low genetic diversity, we found evidence for population admixture. Variation in microsatellite allele frequencies among populations segregated to only a very small extent within mtDNA haplotypes. This is most likely the result of male-biased gene flow between populations of different haplotypes. Male-biased gene flow will not affect the distribution of mtDNA haplotypes, as they are inherited matrilinearly. In guppies, males migrate at a higher rate than females (Croft *et al*. 2003), supporting this interpretation. One female of migrant ancestry was detected among Queensland populations, providing evidence for recent gene flow between populations in the same river system. Low bootstrap support for nodes within phylogenetic trees based on microsatellites indicated that populations were not highly differentiated, except for the cluster of Crystal and Alice. F statistics were also similar across most populations. The low level of population differentiation in microsatellite allele frequencies is consistent with historical gene flow between populations, or of multiple introductions of males. Given the popularity of guppies as aquarium fish, and their widespread distribution in northern Queensland waterways, human-assisted migration or multiple introductions of males cannot be discounted as a source of gene flow. However, as gene flow has not led to a high level of neutral genetic diversity within populations, a likely scenario is that migration events or multiple introductions alternating with bottlenecks have reduced genetic variation. Strong skew in reproductive success (Becher & Magurran 2004) could also have contributed to loss of variation.

Sources for Queensland populations

There are a minimum of two source populations for the seven introduced Australian populations studied, with one in Guyana near the capital, Georgetown, and one in the Caroni drainage, western Trinidad. Both identified sources are near capital cities in countries that were part of the British Empire, which is consistent with a scenario in which the British Colonial Office sent guppies between colonies for mosquito control (Boulenger 1912). Singapore strains also appear to originate from within the Caroni River system, but differences in their mtDNA haplotype to that of Queensland guppies suggests that they are not directly related.

The Guyanese population was genotyped to test the hypothesis that it is an ancestral population to the Queensland populations with which it shares a common mtDNA haplotype, Alligator and Mena. Phylogenetic trees supported relatedness between the Guyana population and Alligator (haplotype 1), while F-tests supported relatedness to Mena (haplotype 1) and Mulgrave (haplotype 2). Although these two approaches differed in which populations were

identified as related to the Guyanese population, each identified one population known to share an mtDNA haplotype with Guyana, providing support for the hypothesis that it is an ancestral population.

Low neutral but high additive genetic diversity

Successful invasive species are generally thought to have high genetic diversity, which allows them to escape the harmful effects of inbreeding (Keller & Waller 2003; Spielman *et al*. 2004) and adapt to their new environment (Sakai *et al*. 2001; Allendorf & Lundquist 2003). Genetic sampling of contemporary populations of guppies in Queensland provide no evidence for high neutral genetic diversity, despite invasion success in Queensland and elsewhere in Australia: Northern Territory (Letts 2004), Western Australia (collections of the Western Australian Museum and the American Museum of Natural History), Norfolk Island, New South Wales (Australian Museum collections) and the territories of Christmas Island and Cocos (Keeling) Island (Western Australian Museum) and many other countries. All seven populations in Queensland had relatively low neutral genetic diversity and only one mtDNA haplotype was detected within each population. Compared to a wild Guyanese population, heterozygosity at nuclear loci averaged 72%, and allelic diversity averaged 37% (Table 6).

The discrepancy between high levels of additive genetic variance in autosomal and Y-linked traits seen in the Alligator population (Brooks & Endler 2001a) and the reduction of neutral genetic diversity and evidence of founder effects and/or bottlenecks reported here may be due to two processes. The first is disruptive selection in combination with negative frequency-dependent selection, which is expected to increase additive genetic variance (Falconer & Mackay 1989). Guppies from Alligator are subject to strong disruptive sexual selection (Blows *et al*. 2003). Male guppies in this population, as in others, are highly variable phenotypically (Brooks & Endler 2001a). Some of this phenotypic variation is maintained by disruptive selection on male ornaments, as there is no single combination of male ornaments that females find attractive (Brooks & Endler 2001a; Blows *et al*. 2003), in combination with negative frequency-dependent selection. Such negative frequency dependence is supported by the presence of female preferences for rare male phenotypes (Farr 1977; Hughes *et al*. 1999).

The second process is the release of additive genetic variation following bottlenecks (Goodnight 1988; Willis & Orr 1993), which has been observed experimentally (e.g. Cheverud *et al*. 1999). With inbreeding coefficients up to $f = 0.5$, some low-frequency recessive alleles become more common through drift, thereby increasing additive variance (Robertson 1952; Willis & Orr 1993). Additive variance can also be increased through conversion of epistatic or dominance genetic variance to additive genetic variance (Meffert 2000). As some loci become fixed, epistatic interactions between loci, and dominance interactions between alleles, may be transmitted as additive effects (Walsh & Lynch 1998; Meffert 2000). However, it is very difficult to predict empirically whether a bottleneck will lead to an increase or a decrease in additive genetic variance (Walsh & Lynch 1998; Barton & Turelli 2004).

Our results reaffirm the difficulty of predicting the response of species to new environments (Allendorf & Lundquist 2003) and to population bottlenecks (Cheverud *et al*. 1999). In the case of guppies, diversity at 10 genetic markers was low and indicated founder effects and/or bottlenecks and was therefore not consistent with the documented high levels of additive genetic variance nor the replicated pattern of successful invasion. Similarly, in a meta-analysis molecular genetic variation did not correlate with heritability (Reed & Frankham 2001). In other taxa, high (e.g. Novak & Mack 1993; Kolbe *et al*. 2004) as well as low levels of neutral genetic diversity (e.g. Tsutsui *et al*. 2000; Colautti *et al*. 2005) characterize invasive populations. Neutral genetic diversity is therefore unlikely to predict the potential for invasion success and adaptation. Guppies, however, meet some of the life history characteristics of introduced fishes that have established successfully in the Great Lakes (Kolar & Lodge 2002), as they grow relatively rapidly and are tolerant of a wide range of salinity and temperatures (Meffe & Snelson 1989). Genetic variation underlying life history traits such as these and the processes generating this variation within populations founded by a small number of individuals are likely to be more important in capturing the potential for invasion success than simple measures of neutral genetic diversity.

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References

Abell EH (1913) *Noxious vermin (mosquitoes) order of Governor in Council, metropolitan area of Brisbane*. Government of Queensland, Brisbane.

Alexander HJ, Breden F (2004) Sexual isolation and extreme morphological divergence in the Cumaná guppy: a possible case of incipient speciation. *Journal of Evolutionary Biology*, **17**, 1238–1254.

Allen GR, Midgley SH, Allen M (2002) *Field Guide to the Freshwater Fishes of Australia*. Western Australian Museum, Perth.

Allendorf FW, Lundquist LL (2003) Introduction: population biology, evolution, and control of invasive species. *Conservation Biology*, **17**, 24–30.

Amos W, Hoelzel AR (1991) Long-term preservation of whale skin for DNA analysis. *Reports of the International Whaling Commission*, **special issue 13**, 99–103.

Barton NH, Turelli M (1989) Evolutionary quantitative genetics: how little do we know? *Annual Review of Genetics*, **23**, 337–370.

Barton NH, Turelli M (2004) Effects of genetic drift on variance components under a general model of epistasis. *Evolution*, **58**, 2111–2132.

Becher SA, Magurran AE (2000) Gene flow in Trinidadian guppies. *Journal of Fish Biology*, **56**, 241–249.

Becher SA, Magurran AE (2004) Multiple mating and reproductive skew in Trinidadian guppies. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **271**, 1009–1014.

Becher SA, Russell T, Magurran AE (2002) Isolation and characterization of polymorphic microsatellites in the Trinidadian guppy (*Poecilia reticulata*). *Molecular Ecology Notes*, **2**, 456–458.

Blows MW, Brooks R, Kraft PG (2003) Exploring complex fitness surfaces: multiple ornamentation and polymorphism in male guppies. *Evolution*, **57**, 1622–1630.

Borthwick T (1923) An anti-mosquito campaign in Adelaide. *Health*, **1**, 259–265.

Boulenger EG (1912) Notes on the breeding of the 'millions' fish (*Girardinus poeciloides*). *Proceedings of the Zoological Society of London*, **1912**, 906–908.

Breden F, Ptacek M, Rashed M, Taphorn D, de Figueiredo CA (1999) Molecular phylogeny of the live-bearing fish genus *Poecilia* (Cyprinodontiformes: Poeciliidae). *Molecular Phylogenetics and Evolution*, **12**, 95–104.

Brooks R, Couldridge V (1999) Multiple sexual ornaments coevolve with multiple mating preferences. *American Naturalist*, **154**, 37–45.

Brooks R, Endler JA (2001a) Direct and indirect sexual selection and quantitative genetics of male traits in guppies (*Poecilia reticulata*). *Evolution*, **55**, 1002–1015.

Brooks R, Endler JA (2001b) Female guppies agree to differ: phenotypic and genetic variation in mate choice behaviour and the consequences for sexual selection. *Evolution*, **55**, 1644–1655.

Cheong L (1996) Overview of the current international trade in ornamental trade in ornamental fish, with special reference to Singapore. *Revue Scientifique et Technique de l'Office International des Epizooties*, **15**, 445–481.

Cheverud JM, Vaughn TT, Pletscher LS *et al.* (1999) Epistasis and the evolution of additive genetic variance in populations that pass through a bottleneck. *Evolution*, **53**, 1009–1018.

Colautti RI, Manca M, Viljanen M *et al.* (2005) Invasion genetics of the Eurasian spiny waterflea: evidence for bottlenecks and gene flow using microsattelites. *Molecular Ecology*, **14**, 1869–1879.

Cooling LE (1912) *Appendix 7: Report on Mosquito-Survey of Eight Selected Areas in Brisbane*, p. 29. Officer of the Commissioner of Public Health, Brisbane.

Croft DP, Albanese B, Arrowsmith BJ *et al.* (2003) Sex-biased movement in the guppy (*Poecilia reticulata*). *Oecologia*, **137**, 62–68.

Dunn KA, McEachran JD, Honeycutt RL (2003) Molecular phylogenetics of myliobatiform fishes (Chondrichthyes: Myliobatiformes), with comments on the effects of missing data on parsimony and likelihood. *Molecular Phylogenetics and Evolution*, **27**, 259–270.

Ellegren H (2000) Microsatellite mutations in the germline: implications for evolutionary inference. *Trends in Genetics*, **16**, 551–558.

Ellstrand NC, Schierenbeck KA (2000) Hybridization as a stimulus for the evolution of invasiveness in plants? *Proceedings of the National Academy of Sciences, USA*, **97**, 7043–7050.

Endler JA (1986) *Natural Selection in the Wild*. Princeton University Press, Princeton, NJ.

Falconer DS, Mackay T (1989) *Introduction to Quantitative Genetics*. Pearson Education, Harlow, Essex.

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*, **164**, 1567–1587.

FAO (2004) *DIAS — Database on introductions of aquatic species*. www.fao.org/figis/servlet/static?dom=root&xml=Introsp/introsp_s.xml. Date modified: 16/5/2003.

Farr JA (1977) Male rarity or novelty, female choice behavior, and sexual selection in the guppy, *Poecilia reticulata* Peters (Pisces: Poeciliidae). *Evolution*, **31**, 162–168.

Felsenstein J (1981) Evolutionary trees from gene frequencies and quantitative characters: finding maximum likelihood estimates. *Evolution*, **35**, 1229–1242.

Felsenstein J (2004) *PHYLIP (Phylogeny Inference Package), Version 3.6*. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle.

Felsenstein J, Churchill GA (1996) A hidden Markov model approach to variation among sites in rate of evolution. *Molecular Biology and Evolution*, **13**, 93–104.

Fernando AA, Phang VPE (1990) Inheritance of the tuxedo and blond tuxedo color pattern phenotypes of the guppy, *Poecilia reticulata*. In: *The Second Asian Fisheries Forum* (eds Hirano R, Hanyu I), pp. 487–490. Asian Fisheries Society, Manila, Philippines.

Froese R, Pauly D (2000) *FISHBASE 2000: concepts, design and data sources*. www.fishbase.org, version 10/2004.

Garza JC, Williamson EG (2001) Detection of reduction in population size using data from microsatellite loci. *Molecular Ecology*, **10**, 305–318.

Goodnight CJ (1988) Epistasis and the effect of founder events on the additive genetic variance. *Evolution*, **42**, 441–454.

Goudet J (2001) *FSTAT version 2.9.3, a program to estimate and test gene diversities and fixation indices*. Available at http://www.unil.ch/izea/softwares/fstat.html.

Grant PR, Grant BR (2000) Quantitative genetic variation in populations of Darwin's finches. In: *Adaptive Genetic Variation in the Wild* (eds Mousseau TA, Sinervo B, Endler JA), pp. 3–40. Oxford University Press, Oxford.

Halliburton R (2004) *Introduction to Population Genetics*. Pearson Education International, Upper Saddle River, NJ.

Hamlyn-Harris R (1929) The relative value of larval destructors and the part they play in mosquito control in Queensland. *Proceedings of the Royal Society of Queensland*, **41**, 23–38.

Herre AWCT (1940) Additions to the fish fauna of Malaya and notes on rare or little known Malayan and Bornean fishes. *Bulletin of the Raffles Museum*, **16**, 27–61.

Houde AE (1994) Effect of artificial selection on male colour patterns on mating preference of female guppies. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **256**, 125–130.

Houde AE (1997) *Sex, Color, and Mate Choice in Guppies*. Princeton University Press, Princeton, NJ.

Hughes KA, Linh Du F, Rodd H, Reznick DN (1999) Familiarity

leads to female mate preference for novel males in the guppy, *Poecilia reticulata*. *Animal Behaviour*, **58**, 907–916.
Keller LF, Waller DM (2003) Inbreeding effects in wild populations. *Trends in Ecology & Evolution*, **17**, 230–241.
Khoo G, Lim TM, Chan WK, Phang VPE (1999) Linkage analysis and mapping of three sex-linked color pattern genes in the guppy, *Poecilia reticulata*. *Zoological Science*, **16**, 893–903.
Kocher TD, Thomas WK, Meyer A *et al.* (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences, USA*, **86**, 6196.
Kolar CS, Lodge DM (2002) Ecological predictions and risk assessment for alien fishes in North America. *Science*, **298**, 1233–1236.
Kolbe JJ, Glor RE, Schettino LR *et al.* (2004) Genetic variation increases during biological invasion by a Cuban lizard. *Nature*, **431**, 177–181.
Lee CE (2002) Evolutionary genetics of invasive species. *Trends in Ecology & Evolution*, **17**, 386–391.
Letts GA (2004) *Feral Animals in the Northern Territory: Report of the Board of Inquiry 1979*. D. W. McDowell, Darwin, Northern Territory.
Lowe A, Harris S, Ashton P (2004) *Ecological Genetics: Design, Analysis and Application*. Blackwell Publishing, Malden, MA.
Luikart G, Cornuet JM (1998) Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. *Conservation Biology*, **12**, 228–237.
McKay RJ (1984) Introductions of exotic fishes in Australia. In: *Distribution, Biology, and Management of Exotic Fishes* (eds Courtenay WR, Stauffer JR), pp. 177–199. Johns Hopkins University Press, Baltimore, MD.
McKay R (1987) It's your problem too! Part 3, The Australian introductions. *Aquarium Life Australia*, **2**, 38–41.
Meffe GK, Snelson FF Jr (1989) An ecological overview of poeciliid fishes. In: *Ecology and Evolution of Live-bearing Fishes* (eds Meffe GK, Snelson FF Jr), pp. 13–31. Prentice Hall, New Jersey.
Meffert LM (2000) The evolutionary potential of morphology and mating behavior: the role of epistasis in bottlenecked populations. In: *Epistasis and the Evolutionary Process* (eds Wolf JB, Brodie ED III, Wade MJ), pp. 177–193. Oxford University Press, Oxford.
Moritz C, Dowling TE, Brown WM (1987) Evolution of animal mitochondrial DNA: relevance for population biology and systematics. *Annual Review of Ecology and Systematics*, **18**, 269–292.
Nei M (1972) Genetic distance between populations. *American Naturalist*, **106**, 283–292.
Nei M, Maruyama T, Chakraborty R (1975) The bottleneck effect and genetic variability in populations. *Evolution*, **29**, 1–10.
Nicholson G, Smith AV, Jónsson F *et al.* (2002) Assessing population differentiation and isolation from single-nucleotide polymorphism data. *Journal of the Royal Statistical Society B*, **64**, 695–715.
Novak SJ, Mack RN (1993) Genetic variation in *Bromus tectorum* (Poaceae): comparison between native and introduced populations. *Heredity*, **71**, 167–176.
Patrick R (1987) *A History of Health and Medicine in Queensland, 1824–1960*. University of Queensland Press, St Lucia.
Phang VPE, Ng LN, Fernando AA (1989) Inheritance of the snakeskin color pattern in the guppy, *Poecilia reticulata*. *Journal of Heredity*, **80**, 393–399.
Piry S, Luikart G, Cornuet JM (1999) BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. *Journal of Heredity*, **90**, 502–503.
Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics*, **14**, 817–818.
Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.
Ptacek MB, Breden F (1998) Phylogenetic relationships among the mollies (Poeciliidae Poecilia: Molliensia group) based on mitochondrial DNA sequences. *Journal of Fish Biology*, **53**, 64–81.
Reed DH, Frankham R (2001) How closely related are molecular and quantitative measure of genetic variation? A meta-analysis. *Evolution*, **55**, 1095–1103.
Robertson A (1952) The effect of inbreeding on the variation due to recessive genes. *Genetics*, **37**, 189–207.
Rosen DE, Bailey R (1963) The poeciliid fishes (Cyprinodontiformes), their structure, zoogeography, and systematics. *Bulletin of the American Museum of Natural History*, **126**, 1–176.
Sakai AK, Allendorf FW, Holt JS *et al.* (2001) The population biology of invasive species. *Annual Review of Ecology and Systematics*, **32**, 305–332.
Seckinger J, Brinkman H, Meyer A (2002) Microsatellites in the genus *Xiphophorus*, developed in *Xiphophorus montezumae*. *Molecular Ecology Notes*, **2**, 4–6.
Shaw PW, Carvalho GR, Seghers BH, Magurran AE (1992) Genetic consequences of an artificial introduction of guppies (*Poecilia reticulata*) in N. Trinidad. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **248**, 111–116.
Shields GF, Kocher TD (1991) Phylogenetic relationships of North American ursids based on analysis of mitochondrial DNA. *Evolution*, **45**, 218–221.
Spielman D, Brook BW, Frankham R (2004) Most species are not driven to extinction before genetic factors impact them. *Proceedings of the National Academy of Sciences, USA*, **101**, 15261–15264.
Taylor JS (1999) *The evolution of repetitive DNA in the guppy (*Poecilia reticulata*) and the genetic structure of natural guppy populations*. PhD thesis, Simon Fraser University, Burnaby, Canada.
Taylor JS, Breden F (2000) Slipped-strand mispairing at noncontiguous repeats in *Poecilia reticulata*: a model for minisatellite birth. *Genetics*, **155**, 1313–1320.
Tsutsui ND, Suarez AV, Holway DA, Case TJ (2000) Reduced genetic variation and the success of an invasive species. *Proceedings of the National Academy of Sciences, USA*, **97**, 5948–5953.
UNCTAD/GATT (1979) *International Trade in Tropical Aquarium Fish*, pp. 1–137. International Trade Centre, Geneva.
Vipan JAM (1910) Malaria and the 'millions' fish (*Girardinus poeciloides*). *Proceedings of the Zoological Society of London*, **1910**, 146–147.
Walsh B, Lynch M (1998) *Changes in variance induced by random genetic drift*. http://nitro.biosci.arizona.edu/zbook/volume_2/chapters/vol2_03.html. Last update 31/7/1998.
Welcomme RL (1988) International introductions of inland aquatic species. *FAO Fisheries Technical Paper*, **294**, 1–318.
Willis JH, Orr HA (1993) Increased heritable variation following population bottlenecks: the role of dominance. *Evolution*, **47**, 949–957.

This project forms part of a larger study on the evolution of sexually selected traits in introduced Australian guppies by Anna Lindholm, Rob Brooks and John Endler. Felix Breden and Heather Alexander (PhD student at Simon Fraser University) use phylogeography as a tool to investigate speciation and trait evolution in poeciliid fishes. Woon-Chan Khiong and Sumita Thakurta are interested in the genetic background of introduced guppies in Singapore.

Chapter 6

Where do all the maternal effects go? Variation in offspring body size throughout ontogeny in the live-bearing fish *Poecilia parae*

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Where do all the maternal effects go? Variation in offspring body size through ontogeny in the live-bearing fish *Poecilia parae*

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Maternal effects are an important source of adaptive variation, but little is known about how they vary throughout ontogeny. We estimate the contribution of maternal effects, sire genetic and environmental variation to offspring body size from birth until 1 year of age in the live-bearing fish *Poecilia parae*. In both the sexes, maternal effects on body size were initially high in juveniles, and then declined to zero at sexual maturity. In sons, this was accompanied by a sharp rise in sire genetic variance, consistent with the expression of Y-linked loci affecting male size. In daughters, all variance components decreased with time, consistent with compensatory growth. There were significant negative among-dam correlations between early body size and the timing of sexual maturity in both sons and daughters. However, there was no relationship between early life maternal effects and adult longevity, suggesting that maternal effects, although important early in life, may not always influence late life-history traits.

Keywords: maternal effects; offspring size; ontogeny; Y-chromosome; *Poecilia parae*

1. INTRODUCTION

An offspring's phenotype is determined not only by its own genotype and the random environmental conditions it experiences during development, but also by the environment provided by its parents (Mousseau & Fox 1998*a*). The environment provided by the mother usually contributes considerably more to offspring phenotype than that by the father, and this asymmetry is generally referred to as a maternal effect (Mousseau & Fox 1998*a*). Recent theoretical (Kirkpatrick & Lande 1989; Cheverud & Moore 1994; Wade 1998) and empirical studies (reviewed in Mousseau & Fox 1998*b*) have demonstrated that maternal effects can have profound evolutionary implications.

Despite their potential importance, there is surprisingly little known about the biology of maternal effects (Mousseau & Fox 1998*a*), particularly their persistence across consecutive developmental stages (Wilson & Réale 2006). A common pattern that emerges from empirical studies is that maternal effects are typically large early in development and far less so by the time offspring mature (Bernardo 1996; Heath & Blouw 1998). This finding strongly suggests that the importance of maternal effects declines throughout offspring development. However, because most empirical studies only quantify maternal effects at birth and adulthood, little is known about the importance of maternal effects relative to other causal components of variance (e.g. additive genetic and environmental effects) throughout development. For example, reductions in maternal effects through ontogeny could arise because the relative importance of either random environmental variation and/or additive genetic variance increases across consecutive developmental stages.

Here, we quantify the relative importance of maternal effects (V_M), sire genetic variation (V_S) and residual environmental variation (V_E) to offspring body size throughout maturation in both the sexes of the live-bearing poeciliid fish *Poecilia parae*. We examine the consequences of these maternal effects on two important life-history parameters: the timing of sexual development and longevity.

2. MATERIAL AND METHODS

(a) *Fish biology and collection*

Poecilia parae is a live-bearing poeciliid fish with internal fertilization. In this genus, offspring develop for 20–30 days within ovarian follicles located inside a vascularized ovary and are independent after birth (Constantz 1989). Wild-caught females produce 4.0±0.3 (mean±s.e.) offspring per brood (n=63, A. K. Lindholm 1999, unpublished). Males are characterized by five colour morphs (red, yellow, blue, parae and immaculata) and an associated size dimorphism (Lindholm *et al.* 2004). Pedigree analysis has demonstrated that the locus or loci controlling this colour polymorphism are linked to the Y-chromosome (Lindholm *et al.* 2004). This mode of inheritance is common in poeciliid fishes, with 13 species known to have Y-linked variation in male colour and/or body size (Lindholm & Breden 2002).

In February 2002, we collected 125 males and 125 females from two sites along the Demerera River in Guyana, South America: 6°41.77′ N; 58°12.07′ W and 6°48.06′ N; 58°09.14′ W. All the fishes were air-freighted to Sydney, Australia, where they were kept at 26°C on a 12 h day : night cycle and fed to satiation on brine shrimp once per day, 6 days per week. Offspring were collected daily and reared individually in 1 l plastic containers. These offspring served as the parents in our breeding design.

(b) *Breeding design*

Our breeding design consisted of 31 sires (four red, six blue, seven yellow, seven parae and seven immaculata), each mated to up to four randomly selected virgin dams. Offspring were collected daily and individually housed in 1 l plastic containers containing 750 ml water and 5 mm gravel, on two adjacent rows of shelves. Containers of newborn offspring were haphazardly stacked, with siblings placed at non-adjacent positions. The positions of containers were then shuffled twice per week to minimize any location effects.

Body size was measured from the anterior tip of the jaw to the posterior end of the caudal peduncle to the nearest 0.5 mm flat against a ruler by the same observer at 2, 4, 6, 8, 12, 16, 20, 28, 36 and 52 weeks of age. Each offspring was visually examined free-swimming in its tank twice per week to assess sexual development. Following Farr & Travis (1986), our index of the onset of sexual maturity was the appearance of pigmented markings on either side of the gonopore in females (see Lindholm *et al.* 2004) and by the differentiation of the anal fin into the gonopodium in males.

Many matings did not produce viable offspring. Moreover, six offspring from five families were born with skeletal curvature, and thus removed from our dataset. In total, 20 sires, 32 dams and 157 offspring (78 daughters and 79 sons) were available for quantitative genetic analysis.

(c) *Statistical analysis*

To estimate V_S, V_M and V_E, we modelled offspring body size as 10 age-specific traits (see Wilson *et al.* 2005) in a nested model

This research was approved by the Animal Care and Ethics Committee of the University of New South Wales.

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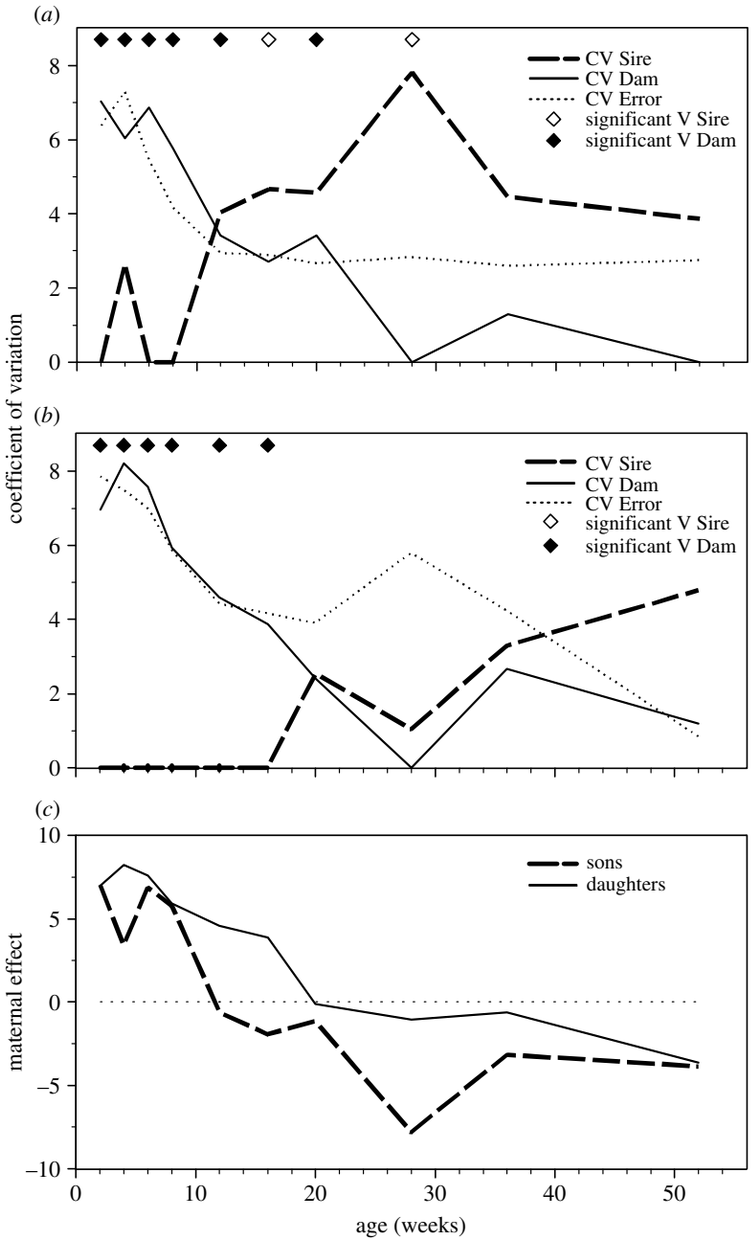

Figure 1. The causal components of variance for offspring body size at each age for (*a*) male and (*b*) female *Poecilia parae*. Variances that are significantly greater than zero at each age are represented by closed diamonds (V_{DAM}) and open diamonds (V_S). (*c*) Maternal effects (CV_M) for offspring body size at each age for sons and daughters.

(sire+dam within sire) using REML in SPSS (v. 12.0, varcomp routine). Among-dam correlations were estimated using ASREML (http://www.vsn-intl.com/ASReml/). In both the cases, significance was assessed using log-likelihood ratio tests. Asymmetries between sire and dam variance components in this kind of design commonly arise when dam estimates are inflated by maternal effects (Lynch & Walsh 1998). Consequently, we use $V_{DAM}-V_S$ as our estimate of maternal effects (V_M; Lynch & Walsh 1998). While this calculation assumes that epistasis and dominance are negligible, studies quantifying maternal effects have shown that they are generally much larger than dominance or epistatic effects, making this assumption reasonable (Cheverud & Moore 1994). Since variance commonly increases with the square of the mean, scale effects may limit the utility of directly comparing the magnitudes of (co)variance components across different ages. We therefore scale variance components by the relevant trait phenotypic mean using the coefficient of variation (CV; Houle 1992).

3. RESULTS

In early development, body size in male (figure 1*a*) and female (figure 1*b*) *P. parae* is strongly influenced by dam variance, which is reflected in the maternal effect (figure 1*c*). In sons, but not daughters, sire variance increased rapidly after eight weeks (figure 1*a*,*b*). In both the sexes, the residual environmental variance decreased during ontogeny. These trends are supported by ANOVA of variance components estimated for each age and sex (table 1). Age of the offspring had a significant effect on all variance components with CV_S showing a significant increase with age, whereas all other variance components (CV_M, CV_E, CV_P) significantly decreased with age (table 1). Moreover, sex of the offspring only influenced levels of CV_S for body size, being significantly greater in sons than in daughters (table 1).

Body size at week 2 was negatively correlated with the index of time to sexual maturity in both sons (mean time to sexual maturity ($\pm$s.e.)$=32.95\pm1.15$ days;

Table 1. The effect of offspring sex and age on the causal components of variation for offspring body size in *Poecilia parae*.

source	factor	$F_{(1,1,1,16)}$	p
CV_S	sex	8.43	0.010
	age	16.95	0.001
	sex×age	0.06	0.813
CV_M	sex	1.57	0.228
	age	27.43	0.001
	sex×age	0.24	0.631
CV_E	sex	1.91	0.186
	age	30.58	0.001
	sex×age	2.92	0.107
CV_P	sex	0.04	0.842
	age	24.72	0.001
	sex×age	0.29	0.596

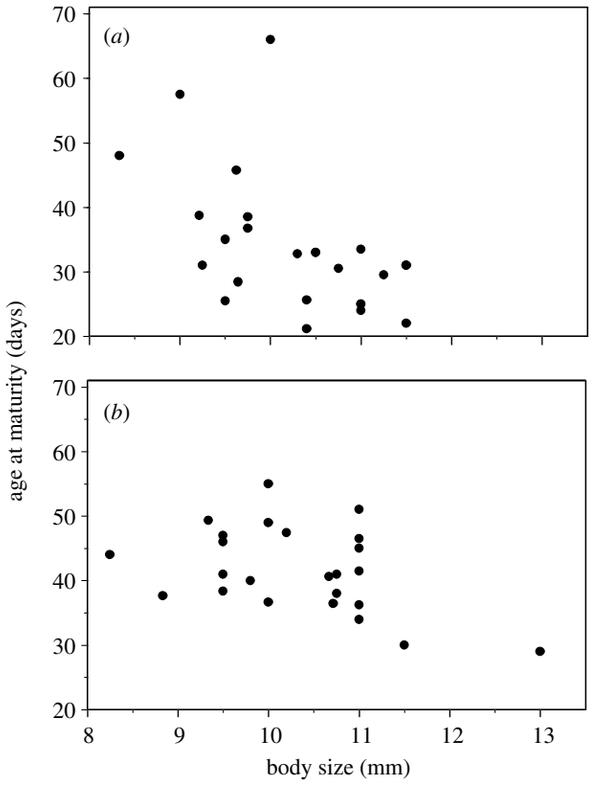

Figure 2. Among-dam correlation between offspring body size at two weeks of age and sexual development in (*a*) male and (*b*) female *Poecilia parae*.

$r_M = -0.43 \pm 0.06$, $p < 0.0001$) and daughters (mean time to sexual maturity = 40.25 ± 0.86 days; $r_M = -0.34 \pm 0.03$, $p < 0.0001$; figure 2). There was, however, no effect of week 2 body size on the longevity of either sons (mean longevity = 332.92 ± 18.25 days; $r_M = 0.03 \pm 0.02$, $p = 0.13$) or daughters (mean longevity = 290.19 ± 12.36 days; $r_M = 0.01 \pm 0.02$, $p = 0.62$).

4. DISCUSSION

Here, we show that maternal effects are a major determinant of early offspring body size in *P. parae* and that they decrease from birth onwards. More importantly, we demonstrate that this decline occurs through different pathways in sons and daughters. Furthermore, while we show that maternal effects may have important consequences for life-history traits expressed early in life (e.g. timing of sexual maturity), they do not influence late life-history traits (i.e. adult longevity). This is consistent with previously found associations between large juvenile body size and early maturation (reviewed in Heath & Blouw 1998). Our results, therefore, demonstrate the complex nature of maternal effects and highlight the relevance of ontogenetic changes when examining the evolutionary consequences of maternal effects (Atchley & Zhu 1997; Heath & Blouw 1998; Wilson & Réale 2006).

Several processes may influence changes in causal variance components during ontogeny (reviewed by Wilson & Réale 2006). In daughters, the declining CV_M, CV_E and CV_P through ontogeny, coupled with negligible levels of CV_S for body size, is largely consistent with compensatory growth (Wilson & Réale 2006)—the tendency of growth trajectories to converge on a reduced range of phenotypes (Monteiro & Falconer 1966). Compensatory growth is an important mechanism of canalization that buffers the adult phenotype against stressors encountered during early life (Monteiro & Falconer 1966) and appears widespread among vertebrates (Wilson & Réale 2006). As adults, female poeciliids grow much faster than males (Snelson 1989), which would provide greater scope for growth compensation to occur. The dramatic increase of CV_S observed in sons, by contrast, probably results from the activation of the Y-chromosome (or associated loci) at about sexual maturity. Similar sex-specific differences in inheritance of body size have been found in other poeciliid species (Kallman 1989).

We quantify maternal effects as the difference between sire and dam variances. Although this is a commonly used measure (see Lynch & Walsh 1998), it assumes that dominance and epistasis variances are negligible. While the allocation of maternal resources to offspring during the formation of yolked eggs and gestation are well known in poeciliids (Constantz 1989), it is possible that our estimates of CV_M are inflated by dominance and/or epistasis and affected by the relatively small size of the dataset.

Recently, there has been an increase in the number of studies examining the evolutionary consequences of maternal effects (Mousseau & Fox 1998*a*). Most studies are, however, phenotypic and only characterize maternal effects at a single point in time, often independent of other variance components. This is surprising, given that our ability to make quantitative genetic predictions about phenotypic evolution is based on such variance components remaining constant across consecutive developmental stages (Lynch & Walsh 1998). Moreover, the evolutionary consequences of maternal effects are seldom examined across different life-history stages. Our finding that maternal effects may have important consequences for life-history traits expressed early in life (i.e. the timing of sexual maturity), but these effects may not persist to later life-history stages (i.e. adult longevity), demonstrates the potential limitations of such an approach and highlights the need for longitudinal studies when examining the adaptive significance of maternal effects.

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Atchley, W. R. & Zhu, J. 1997 Developmental quantitative genetics, conditional epigenetic variability and growth in mice. *Genetics* **147**, 765–776.

Bernardo, J. 1996 Maternal effects in animal ecology. *Am. Zool.* **36**, 83–105.

Cheverud, J. M. & Moore, A. J. 1994 Quantitative genetics and the role of the environment provided by relatives in behavioral evolution. In *Quantitative genetic studies of behavioral evolution* (ed. C. R. B. Boake), pp. 67–100. Chicago, IL: University of Chicago Press.

Constantz, G. D. 1989 Reproductive biology of poeciliid fishes. In *Ecology and evolution of livebearing fishes (Poeciliidae)* (ed. G. K. Meffe & F. F. J. Snelson), pp. 33–50. Englewood Cliffs, NJ: Prentice Hall.

Farr, J. A. & Travis, J. 1986 Fertility advertisement by female sailfin mollies, *Poecilia latipinna* (Pisces: Poeciliidae). *Copeia* **1986**, 467–472. (doi:10.2307/1445004)

Heath, D. D. & Blouw, D. M. 1998 Are maternal effects in fish adaptive or merely physiological side effects? In *Maternal effects as adaptations* (ed. T. A. Mousseau & C. W. Fox), pp. 178–201. New York, NY: Oxford University Press.

Houle, D. 1992 Comparing evolvability and variability of quantitative traits. *Genetics* **130**, 195–204.

Kallman, K. D. 1989 Genetic control of size at maturity in *Xiphophorus*. In *Ecology and evolution of livebearing fishes (Poeciliidae)* (ed. G. K. Meffe & F. F. Snelson), pp. 163–184. New Jersey, NJ: Prentice Hall.

Kirkpatrick, M. & Lande, R. 1989 The evolution of maternal characters. *Evolution* **32**, 485–503. (doi:10.2307/2409054)

Lindholm, A. & Breden, F. 2002 Sex chromosomes and sexual selection in poeciliid fishes. *Am. Nat.* **160**, S143–S224. (doi:10.1086/342898)

Lindholm, A. K., Brooks, R. & Breden, F. 2004 Extreme polymorphism in a Y-linked sexually selected trait. *Heredity* **92**, 156–162. (doi:10.1038/sj.hdy.6800386)

Lynch, W. & Walsh, B. 1998 *Genetics and analysis of quantitative traits*. Sunderland, MA: Sinauer Associates.

Monteiro, L. S. & Falconer, D. S. 1966 Compensatory growth and sexual maturity in mice. *Anim. Prod.* **8**, 179–192.

Mousseau, T. A. & Fox, C. W. 1998*a* The adaptive significance of maternal effects. *Trends Ecol. Evol.* **13**, 403–407. (doi:10.1016/S0169-5347(98)01472-4)

Mousseau, T. A. & Fox, C. W. (eds) 1998 *Maternal effects as adaptations*. Oxford, UK: Oxford University Press.

Snelson, F. F. J. 1989 Social and environmental control of life history traits in poeciliid fishes. In *Ecology and evolution of live-bearing fishes (Poeciliidae)* (ed. G. K. Meffe & F. F. J. Snelson), pp. 149–161. Englewood Cliffs, NJ: Prentice Hall.

Wade, A. J. 1998 The evolutionary genetics of maternal effects. In *Maternal effects as adaptations* (ed. T. A. Mousseau & C. W. Fox), pp. 5–21. Oxford, UK: Oxford University Press.

Wilson, A. J. & Réale, D. 2006 Ontogeny of additive and maternal genetic effects: lessons from domestic mammals. *Am. Nat.* **167**, E23–E38. (doi:10.1086/498138)

Wilson, A. J., Kruuk, L. E. B. & Coltman, D. W. 2005 Ontogenetic patterns in heritable variation for body size: using random regression models in a wild ungulate population. *Am. Nat.* **166**, E177–E192. (doi:10.1086/497441)

Chapter 7

Causes of male sexual trait divergence in introduced populations of guppies

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Causes of male sexual trait divergence in introduced populations of guppies

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Keywords:

alignment;
coloration;
genetic drift;
introduced populations;
natural selection;
Poecilia reticulata;
population divergence;
predation;
selection gradient;
sexual selection.

Abstract

Males from different populations of the same species often differ in their sexually selected traits. Variation in sexually selected traits can be attributed to sexual selection if phenotypic divergence matches the direction of sexual selection gradients among populations. However, phenotypic divergence of sexually selected traits may also be influenced by other factors, such as natural selection and genetic constraints. Here, we document differences in male sexual traits among six introduced Australian populations of guppies and untangle the forces driving divergence in these sexually selected traits. Using an experimental approach, we found that male size, area of orange coloration, number of sperm per ejaculate and linear sexual selection gradients for male traits differed among populations. Within populations, a large mismatch between the direction of selection and male traits suggests that constraints may be important in preventing male traits from evolving in the direction of selection. Among populations, however, variation in sexual selection explained more than half of the differences in trait variation, suggesting that, despite within-population constraints, sexual selection has contributed to population divergence of male traits. Differences in sexual traits were also associated with predation risk and neutral genetic distance. Our study highlights the importance of sexual selection in trait divergence in introduced populations, despite the presence of constraining factors such as predation risk and evolutionary history.

Introduction

Sexual selection is an important evolutionary process in natural populations and is often stronger than other forms of natural selection (Kingsolver *et al.*, 2001; Hoekstra *et al.*, 2002; Kingsolver & Pfennig, 2007; Svensson & Gosden, 2007). Variation among geographically isolated conspecific populations in sexual advertisement, mate choice and sexual behaviour is important because resulting differences in the direction and intensity of sexual selection may drive divergence in sexually selected and other correlated traits. Furthermore, covariation between male sexual advertisement and female preferences for those advertisements provides evidence that sexual selection can determine the direction and strength of evolutionary diversification (e.g. in orthopterans, *Ephippiger ephippiger*, Ritchie, 1991; house finches *Carpodacus mexicanus frontalis*, Hill, 1994; and frogs, *Physalaemus petersi*, Boul *et al.*, 2007). Thus, an examination of the patterns of interpopulation variation and covariation of sexual traits and sexual selection on those traits as well as the processes underlying

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divergence can help us to understand sexual selection, the evolution of mate choice and the potential for these processes to influence speciation (but see Claridge & Morgan, 1993; Houde, 1993; Verrell, 1999; Boughman, 2001).

Sexual selection is not the only process that influences the diversification of sexual traits. Other forms of selection can also impact sexually selected traits and preferences, which may lead to population-dependent trajectories of trait evolution that do not align with differences in sexual selection. For instance, antagonistic interactions between sexual and other forms of natural selection have been shown to influence the co-evolution of ornaments and preferences (Schwartz & Hendry, 2007; Gordon *et al.*, 2009; Weese *et al.*, 2010). Predation, in particular, exerts natural selection on both sexual advertisement traits and preferences for those traits. For example, sexually preferred males bearing exaggerated ornaments are also more conspicuous to predators (Endler, 1980; Magnhagen, 1991; Godin & McDonough, 2003; Millar *et al.*, 2006; Schwartz & Hendry, 2007). Net sexual selection can thus be weaker in the presence of predators (Schwartz & Hendry, 2007; Weese *et al.*, 2010). Indeed, predictable relationships between nonsexual and sexual selection like these can lead to parallel evolution of sexual traits and mate preferences as seen, for example, in guppies *Poecilia reticulata*, where males from low-predation sites developed larger body sizes and increasing coloration compared to males from high-predation sites, whereas females at high-predation sites discriminated against colourful males (Schwartz & Hendry, 2007). Similarly, in *Drosophila serrata*, changes in cuticular hydrocarbons (CHCs) induced by novel environments also led to divergence of female preferences for these CHCs among populations (Rundle *et al.*, 2005).

Genetic architecture can also influence the rate and direction at which sexual traits and preferences for these traits can evolve, leading to complex interactions between different forms of selection. For example, in an artificial selection experiment on male attractiveness in *Drosophila serrata* (Hine *et al.*, 2011), selection on a preferred trait led to high mating success, but only until an evolutionary limit had been reached. In other words, genetic constraints prevented the unlimited evolution of male sexual traits in the direction of sexual selection. Furthermore, the results highlighted the importance of the interplay between sexual and nonsexual fitness for the evolution of sexual traits and indicated that sexual selection alone (without additional factors such as changes in the environment or changing female preference) is unlikely to drive trait divergence.

The genetic variance–covariance matrix underlying sexual traits and mate preferences may influence the trajectory of trait divergence regardless of how selection operates (Harvey & Pagel, 1991; Schluter, 2000), and may lead to a mismatch between observed ornamental traits and sexually selected optima (Hine *et al.*, 2011). The strong influence of genetic constraints on the direction of divergence in sexually selected traits has been highlighted in a study examining nine *Drosophila serrata* populations (Chenoweth *et al.*, 2010). Chenoweth *et al.* found that sexual selection alone could only account for 10% in population divergence in male CHCs, due to the fact that genetic variation in male CHCs in the direction of sexual selection was low. The evolution of CHCs followed the axes of genetic variance rather than the direction of sexual selection.

Another important factor that may influence evolutionary trajectories is evolutionary history. However, as colonizing populations are often small, and subject to founder effects or bottlenecks, evolutionary change may also result from genetic drift and inbreeding, leading to the loss of genetic variation. Alternatively, interactions between genotype and the new environment, as well as the mixing of genetic variation, when individuals from multiple source populations are introduced to the new site can result in increased additive genetic variance and new patterns of multivariate genetic covariation (Kolbe *et al.*, 2004), which can have strong effects on the direction of evolutionary change post-introduction. Untangling these contributions to trait evolution should lead to a better understanding of both trait divergence and biological invasions.

Our aim in this study was to identify the factors that cause population divergence in male sexual traits. Specifically, we are interested in the roles of selection, drift and constraints. Replicated species introductions provide excellent opportunities to do this. Colonization of new habitats often leads to rapid trait divergence due to adaptation to novel selective environments (Arnold *et al.*, 2001; Reznick & Ghalambor, 2001; Hendry *et al.*, 2008). Previous studies of experimental introductions (Losos *et al.*, 1997; Reznick *et al.*, 1997) show that they can lead to predictable, rapid evolutionary diversification that may parallel diversification seen in native ranges. For example, transplantations of guppies, from different source populations to previously unoccupied neighbouring streams, led to the evolution of male traits along the trajectories allowed by differing predation regimes, taking the differences of the source populations in male traits into account (Endler, 1980). In contrast, other examples show diversification along different trajectories in introduced compared to source populations, such as in house sparrows *Passer domesticus*, which were introduced to North America from Europe and evolved latitudinal clines in body size which were opposite in direction to the clines in Europe (Johnston & Selander, 1973).

In our study, we examine male sexual trait evolution in populations of guppies introduced to Australia, a species known to show geographical covariation between

male advertisement and female choice among naturally occurring populations. Among populations, there are complex multivariate differences in male ornamentation and in mating preferences (Endler, 1990, 1995; Houde & Hankes, 1997). The area of orange coloration and the number of black spots are each positively correlated with the strength of preferences that females express for these traits in natural populations (Houde & Endler, 1990; Endler & Houde, 1995). Conspicuous colour patterns are also associated with the incidence of visual predators (Endler, 1987; Schwartz & Hendry, 2007). Together, these studies provide empirical support for a match between the signalling environment, male display and female mate choice. However, the match between male display and female preference in guppies is neither perfect nor universal. For example, a comparison of female preferences between two populations differing strongly in male orange area found no differences in levels of female sexual responsiveness or orange preference functions (Houde & Hankes, 1997).

The fastest diversification of sexually selected traits ever observed in natural populations is that of male coloration in guppies (Endler, 1980; Svensson & Gosden, 2007). Life history traits in guppies can also evolve very rapidly (Reznick *et al.*, 1990) in response to altered selection when introduced to new streams within Trinidad and especially in response to modified predation regime (Millar *et al.*, 2006; Schwartz & Hendry, 2007; Gordon *et al.*, 2009). Feral guppy populations have become established in hundreds of natural water bodies around the world, due both to their proliferation as pets and to their perceived usefulness in mosquito control (Lindholm *et al.*, 2005). In North Queensland, Australia, several known introductions of guppies have occurred since 1910 (Lindholm *et al.*, 2005).

To document interpopulation variation in both male sexual traits and sexual selection on these traits, we collected males and females from six introduced populations and measured sexual selection in each of these populations in laboratory trials, using paternity analysis. We predicted that if sexual selection was important in determining interpopulation variation in male sexual traits, then observed divergence in these traits would covary with the direction of sexual selection gradients (Chenoweth *et al.*, 2010). We also tested whether predator-induced natural selection or genetic drift was associated with interpopulation variation in male display traits. If natural selection has been important in the divergence of male sexual traits, then we predicted that interpopulation variation would be associated with important ecological parameters such as predation intensity (measured here as the presence or absence of piscivorous fish). Alternatively if genetic drift is important in determining interpopulation variation in sexual traits, then we expected associations with either genetic (measured from population divergence at neutral genetic markers) or geographical distance.

Materials and methods

Adult male and female guppies were collected with permission from the Queensland Parks and Wildlife Services (Scientific Purposes permit F1/000428/01/SAA) using a dip-netting technique while wading in shallow water near the shore, consistently at all populations. Fish were collected from 5–12 April 2002 at the following sites in North Queensland, Australia: Alligator Creek ('Ack', 19.45°S, 146.97°E), Big Crystal Creek ('Crc', 18.98°S, 146.23°E), Mena Creek ('Mnc', 17.65°S, 145.97°E), the pond at the base of Millaa Millaa Falls ('Mlm', 17.50°S, 145.62°E), Mulgrave River ('Ulg', 17.12°S, 145.45°E) and Wadda Creek ('Wdd', 17.60°S, 145.83°E) (Fig. 1). These populations stem from a minimum of two female source populations introduced at nonadjacent locations (Lindholm *et al.*, 2005). It is unknown when the populations originated, but guppies were first introduced into northern Queensland around 1910 (Lindholm *et al.*, 2005). The guppies were air-transported to Sydney, and populations were housed in separate large, widely spaced tanks in a greenhouse at the University of New South Wales. All fish were maintained on natural daylight schedules and fed live brine shrimp 5 days per week. All methods used in this experiment were approved by the UNSW Animal Care and Ethics Committee (clearance number 00/109).

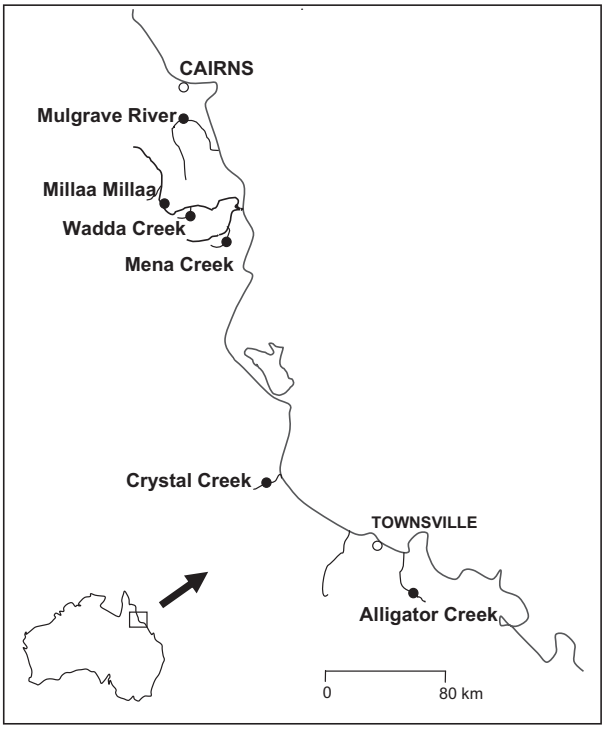

Fig. 1 Sampling locations (black dots) of six feral guppy populations in northern Queensland, Australia

Mating trials and measuring male traits

Two weeks before mating trials commenced, males were removed in groups of ten. Each male was anaesthetized by immersion in a slurry of ice and photographed on its right side with a Nikon Coolpix 950 (Nikon, Tokyo, Japan) digital camera and fin-clipped at the distal end of the tail fin for the isolation of DNA. Males were then housed in individual tanks for 2 weeks, by which time tail fins had regrown. Sperm samples were taken on this day following the methods of Mathews *et al.* (Mathews *et al.*, 1997). Estimates of sperm number were square-root-transformed for parametric analysis.

Males were added in their groups of ten to 200-L aerated plastic tubs. Each tub was lined with gravel, decorated with plastic plants and two cinder blocks. On the day after males were added, ten females from the same population were weighed and measured and introduced to the tank, giving an equal sex ratio. We intended to set up three replicate mating tanks per population, but deaths in some populations limited us to two mating trials for Mulgrave River fish and to a third trial of only eight pairs from Millaa Millaa and five pairs from Mena Creek. After adding the females, we removed and discarded all offspring born for the next 5 weeks. As guppies are typically born after three or 4 weeks of gestation (Houde, 1997), any offspring born within this period are likely to have resulted from a brood cycle started before the mating trial. We then transferred females to individual tanks and waited for them to produce offspring. This allowed us to unambiguously match offspring to mother. Offspring were captured on their day of birth, killed and preserved in 70% ethanol until DNA extraction. After producing their first brood, or a minimum of three offspring, females were fin-clipped for DNA extraction.

Photographs of males were analysed using MeasureMaster Software (version 3.44 (+), 1999 Leading Edge Pty Ltd, Adelaide, Australia) and a digitization tablet. The areas of the body and tail were first measured, and then the areas of the body covered by black, fuzzy black, orange and total iridescence were measured, following the standard protocol (e.g. Head, 2005).

Paternity analyses

DNA was isolated from all mothers, three of their offspring and all potential fathers, by salt precipitation, using Puregene Tissue Kit (Gentra, Gentra Systems, Minneapolis, MN, USA). Nine fluorescently labelled polymorphic microsatellite loci were amplified and scored using Genemapper software (Applied Biosystems, Foster City, CA, USA): TCTG and sat4 (Taylor, 1999), TTA (Taylor, 1999 with redesigned primers), KonD6, KonD15 and KonD21 (Seckinger *et al.*, 2002), Pr39 and Pr80 (Becher *et al.*, 2002) and Pr67 (Becher & Magurran, 2004). The average number of alleles per locus was 4.5 ± 0.25 (SE) per population.

Parent and offspring genotypes were analysed using Cervus 3.0 (Marshall *et al.*, 1998). As Cervus simulations assume Hardy–Weinberg equilibria and linkage equilibrium (Marshall *et al.*, 1998), we tested parental genotypes and found no deviation from Hardy–Weinberg expectations and no linkage disequilibrium, using Genepop web version 3.4 (http://wbiomed.curtin.edu.au/genepop/). Paternity analyses with one known parent were performed using the criteria of number of candidate sires equalling the number of males in a trial, proportion of candidate sires sampled equalling 1, allowing a 1% error rate, the observed proportion of loci typed (ranging from 0.99 to 1.0) and a 80% level of confidence.

Population variation in male traits

To determine whether male phenotypes differed between the populations, we used multivariate analysis of variance (MANOVA). Each male trait (four colour traits, sperm number, body and tail size, $N = 7$) was included as a response variable, and population was included as fixed factor.

Population variation in sexual selection

For selection analyses, relative fitness was calculated as individual fitness (number of offspring sired by a given male) divided by mean fitness (average number of offspring per male) within each trial, and the seven male traits (outlined above) were standardized to the experiment-wide mean and standard deviation (Lande & Arnold, 1983) to allow comparison of the strength of selection both across different traits and across the different populations. To determine whether populations differed in linear sexual selection on male traits, we used a sequential model building approach (Draper & John, 1988). First, we fitted an ANCOVA model containing population as a fixed effect and the linear effects of each of the male traits under investigation as covariates. This model was then compared to a model to which we added linear covariate by population interactions. We determined whether the addition of these interaction terms significantly improved the fit of the model using a partial *F*-test (Bowerman & O'Connell, 1990). When the addition of the interaction terms significantly improves the fit of the model, this indicates that linear sexual selection differs between the populations. We did not calculate nonlinear selection gradients because of sample size limitations.

Within-population alignment of male trait variation and sexual selection

Knowing that both male traits and sexual selection differed between the populations, we wanted to establish whether sexual selection was driving population

divergence in male traits and what other factors might be implicated in constraining the response of male traits to selection. To do this, we first calculated linear selection (β) vectors for each population. The linear selection vector for a given population is a vector of the seven linear selection gradients obtained from a multiple linear regression model (with relative fitness as the response variable and the seven mate traits as predictor variables). To look at the alignment of male trait variation with the direction of sexual selection within each population, we calculated the angle, θ, between the vector of directional selection, (β), and the vector of population mean values for the seven male traits for each pairwise comparison of populations using the following equation:

$$\theta = \cos^{-1}\left(\frac{a \cdot b}{\|a\|\|b\|}\right) \quad (1)$$

This was calculated separately for each population by substituting the vectors of interest into equation (1), such that a is the vector of population trait means and b is the vector of directional selection for a given population. To calculate 95% confidence intervals around each angle estimate, the relative fitness data were randomized within each population, and the selection gradients were re-estimated from the multiple regression models described above. This was repeated 1000 times to generate a distribution of 1000 angle estimates, from which a confidence interval was calculated. This angle, θ, gives a measure of how well-aligned selection and phenotypic variation are within each population. The directionality of the phenotypic vector in each of these calculations is not meaningful, and so we interpreted an angle of 90° as the maximum constraint, where the vector of selection is rotated orthogonal to the phenotypic vector, suggesting that there might be some form of constraint within that population preventing male traits from evolving in the direction of selection. For ease of interpretation, angles between 90° and 180° are represented as the equivalent angle between 0° and 90°.

Among-population covariation in divergence of male traits and sexual selection

To compare population divergence in male traits with divergence in sexual selection, we followed the methods of Chenoweth *et al.* (2010). First, we created a **D** matrix which estimated the variance–covariance matrix among the six population means for each of the seven male traits. We then used the selection gradients (β) for each population obtained from the selection analysis described above to create a **B** matrix, which represents the variance–covariance matrix among the six population selection gradients (β) for each of the seven male traits.

To compare the orientation of these two matrices, we used the Krzanowski method (Krzanowski, 1979). This method required a principal component analysis of each matrix to determine the number of principal components needed to explain most of the variation in each matrix. Only principal components that had eigenvalues greater than one were used. This gave us two principal components for both matrices which in both cases explained over 90% of the variance. These two dimensional subspaces were then compared using equation (5) from Chenoweth *et al.* (2010). All analyses were conducted in R 2.15.0 (R Development Core Team, 2012).

Identification of Predation regimes

We recorded fish species present at each of the collection sites at the time of collection (see also Head, 2005) and during snorkelling. These recordings revealed a total of eleven fish species present at our study sites, of which only three were considered to be potential predators of adult guppies (assessed blind to origin by J.A. Endler with the help of B. Pusey). Observed potential predators were the marbled eel (either *Anguilla obscura* or *Anguilla reinhardtii*), jungle perch (*Kuhlia rupestris*) and mangrove jack (*Lutjanus argentimaculatus*). *Anguilla* sp. and *K. rupestris* were recorded at both the Alligator and Crystal Creek sites, and *L. argentimaculatus* was also recorded at Crystal Creek. A large proportion of the diet of these species comprises small fish comparable in size to guppies (Pusey *et al.*, 2004). None of the predatory species were recorded at the remaining four sites. Due to our noninvasive sampling techniques, we cannot exclude the presence of predatory species at the sites that were classified as 'no predation'; however, we believe that our sampling regime does provide a reliable estimate of relative predation intensity.

The role of genetic and geographical distance in determining population variation in male traits and sexual selection

To determine whether genetic or geographical distance could account for any variation between populations in male traits, we employed a matrix comparison approach often used in population genetic studies (Geffen *et al.*, 2004; Ramachandran *et al.*, 2005). We also investigated whether the variation between populations in sexual selection itself was related to genetic or geographical distance. To do this, we calculated matrices of genetic and geographical distances within Genalex 6.2 (Peakall & Smouse 2006). We report the results based on Nei's genetic distance, but an analysis based on F_{ST} gives very similar results (not shown). Linear geographical distances were calculated based on latitude and longitude coordinates; these were highly correlated with estimated waterway distances (Spearman's rho = 0.94).

We also calculated Euclidean distance matrices for male traits (using standardized trait population means) and sexual selection gradients (using population linear selection gradients for each trait obtained from the above selection analysis). The correlations between these matrices and their significance were calculated using Mantel tests (Sokal & Rohlf, 1995) in PopTools (Excel add-on). Significant correlations between male trait distance and genetic distance or geographical distance may act to constrain male response to sexual selection. On the other hand, significant correlations between sexual selection distance and genetic distance or geographical distance may indicate that genetic background limits the potential for sexual selection to drive trait divergence.

The role of predation regime in determining population variation in male traits and sexual selection

Ecological differences between the populations may be important in determining the relationship between sexual selection and trait divergence. Predation regime has previously been shown to be important in shaping male traits that are also targeted by sexual selection (Endler, 1980; Houde, 1997; Ruell *et al.*, 2013). To test whether population differences in male traits or sexual selection on these traits were associated with population predation regime, we conducted analysis of variance, testing the effect of predation regime on each of the male traits measured (pooled to population means) as well as on each selection gradient associated with each of these traits. To control for the potential for increased type I error that is associated with conducting multiple tests, we calculated corrected *P*-values using the false discovery rate method proposed by Benjamini & Hochberg (1995).

Results

Paternity analyses

The proportion of males that were successful in siring offspring within a trial did not differ between the populations (binary GLM, $z = 0.895$, d.f. = 1.15, $P = 0.37$). The males siring offspring per trial ranged from 50% to 100%.

Male traits vary among populations

Multivariate analysis of variance (MANOVA) revealed that male traits differed significantly among populations (Wilk's lambda = 0.386, $F_{5,157} = 4.633$, $P < 0.001$). Univariate analyses showed that these differences were due to large differences in body and tail size, as well as the area of orange coloration and sperm number (body area in mm^2: $F_{5,157} = 5.663$, $P < 0.001$, tail area in mm^2: $F_{5,157} = 11.632$, $P < 0.001$, orange area in mm^2: $F_{5,157} = 3.783$, $P = 0.003$, sperm number: $F_{5,157} = 3.748$, $P = 0.003$, see also Fig. 2).

Sexual selection differs among populations

There were significant differences among populations in linear sexual selection (partial *F*-test: $F_{7,115} = 4.947$, $P < 0.001$). Linear sexual selection gradients, β, for the seven traits in each of the six populations are given in Table 1 and in Fig. S1.

Within-population alignment of male trait variation and sexual selection

For each of the six populations, the angle between sexual selection and phenotypic vectors was greater than 50°, indicating that there is some form of constraint acting within each population (see Fig. 3). By looking at the overlap of the 95% confidence intervals, we can see that the populations formed two groups with respect to the degree of alignment between sexual selection and phenotypic variation. Selection and phenotypic variation were most closely aligned within the Alligator Creek, Millaa Millaa Falls, Mena Creek and Wadda Creek populations (Fig. 3), whereas Big Crystal Creek and Mulgrave River had significantly weaker alignment, with intervals overlapping the absolute constraint of 90°. These results suggest that the populations differ in terms of constraints on the evolution of these male traits.

Among-population covariation in divergence of male traits and sexual selection

The pattern of population divergence in male traits represented by the **D** matrix (Table S1) was explained by two principal components (eigenvectors d_{max} and d_2, Table 2) that together accounted for 95.2% of the variation among population mean phenotypes. d_{max} contrasted body area, tail area and fuzzy black coloration with black coloration and sperm number (Table 2). Similarly, most (90.1%) of the between-population variation in sexual selection represented in **B** (Table S2) was also accounted for by variation in two principal components (b_{max} and b_2). A comparison of the major subspaces of these two matrices indicated substantial similarity in orientation between them ($\Sigma\lambda_{S(B,D)} = 1.076$ of a possible 2, or 53.8% of the maximum).

The role of predation regime in determining population variation in male traits and sexual selection

The amount of male orange coloration was influenced by the predation regime of the population of origin, whereas males from populations where predators had

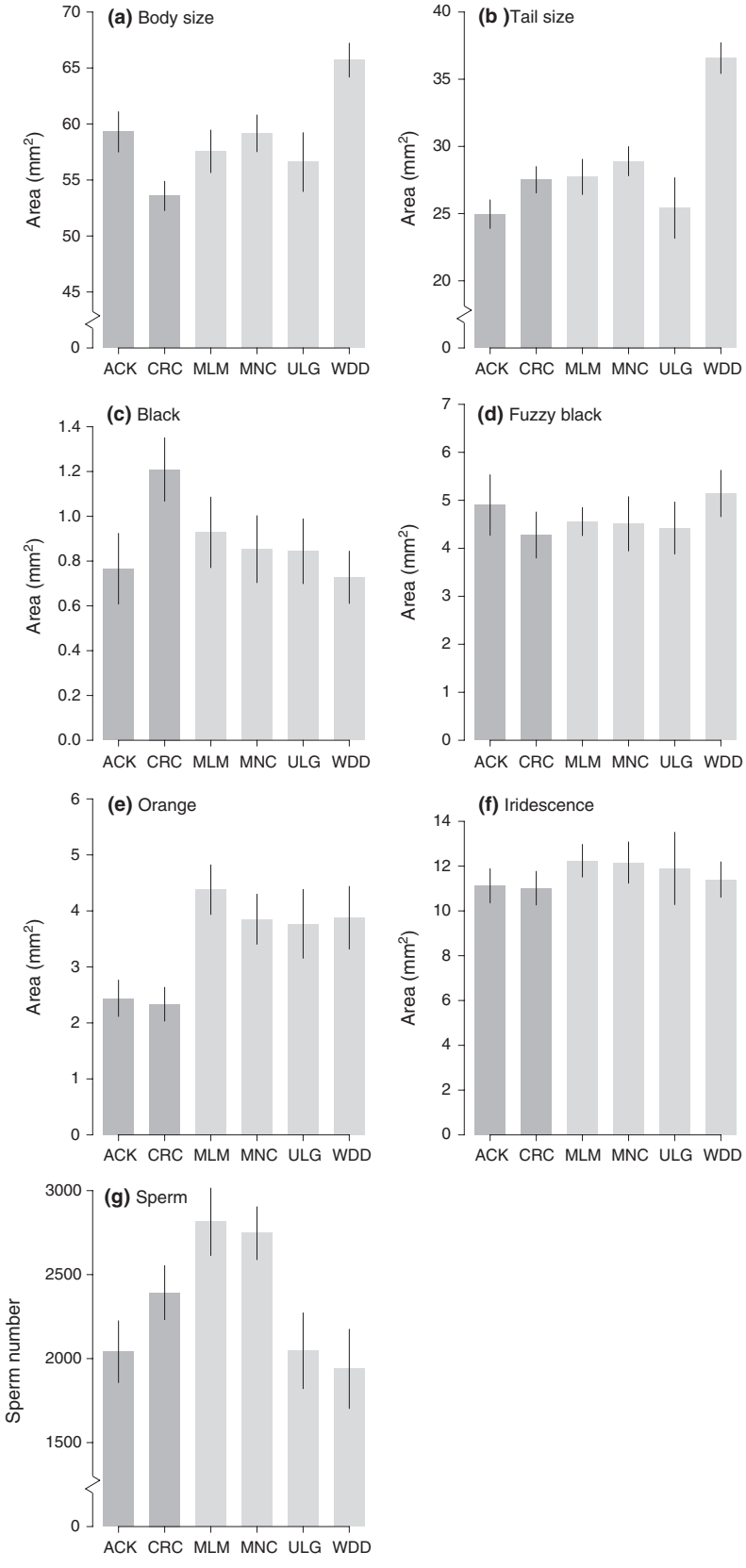

Fig. 2 Population means for the male traits measured in the feral guppy populations (acronyms are explained in the main text): (a) body size (body area), (b) tail size (tail area), (c) area of black coloration, (d) area of 'fuzzy' black coloration, (e) amount of orange ornamentation, (f) area of iridescent coloration, (g) sperm number. Dark grey shaded populations indicated that predators had been found, whereas the light grey shaded populations have been classified as 'no predation'. Note that only orange coloration and iridescent coloration were significantly affected by predation and that this remained stable after correction for multiple comparisons for orange coloration only (see Table 3 for details).

Table 1 Linear selection gradients (β) ± SE for each of the seven male traits within each population. Selection gradients in boldface were significant.

	ACK	CRC	MLM	MNC	ULG	WDD
Body	0.322 ± 0.345	−0.888 ± 0.519	**−1.340** ± 0.564	−0.459 ± 0.803	0.168 ± 0.728	0.051 ± 0.524
Tail	**−1.443** ± 0.426	0.267 ± 0.478	0.587 ± 0.644	−0.123 ± 0.740	−0.887 ± 0.707	0.467 ± 0.392
Black	0.184 ± 0.245	0.240 ± 0.219	0.125 ± 0.256	0.526 ± 0.397	−0.760 ± 0.530	−0.378 ± 0.379
Fuzzy	0.003 ± 0.227	0.083 ± 0.283	−1.260 ± 0.825	−0.094 ± 0.379	0.522 ± 0.534	0.020 ± 0.323
Orange	0.358 ± 0.381	−0.080 ± 0.413	**0.687** ± 0.266	0.484 ± 0.482	0.706 ± 0.388	0.106 ± 0.231
Iridescence	0.316 ± 0.364	−0.089 ± 0.363	**1.221** ± 0.514	0.244 ± 0.483	0.404 ± 0.464	−0.107 ± 0.369
Sperm	−0.139 ± 0.268	−0.088 ± 0.282	0.279 ± 0.244	0.487 ± 0.515	0.701 ± 0.543	0.021 ± 0.263

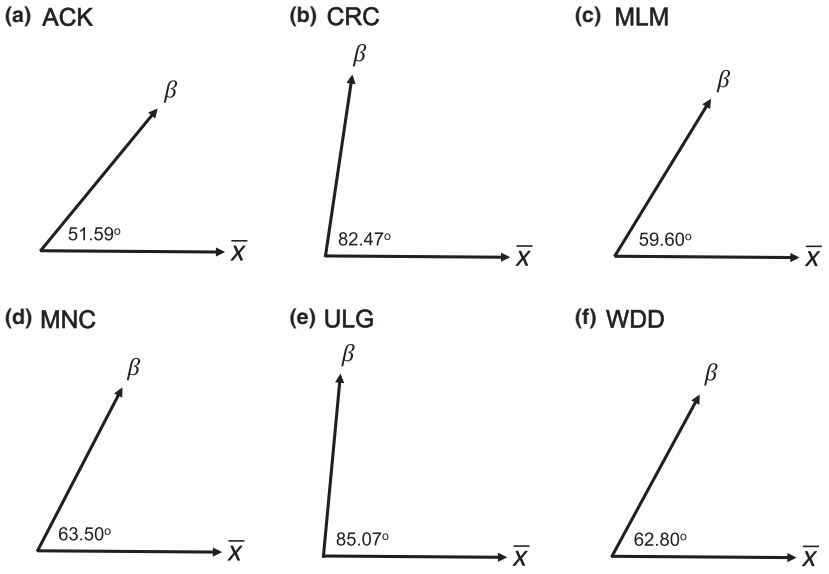

Fig. 3 The alignment between phenotypic variation and sexual selection for each of the feral guppy populations, where large angles represent a greater mismatch between trait variation and the direction of sexual selection compared to smaller angles. (a) Alligator Creek , (b) Big Crystal Creek, (c) Mena Creek, (d) Millaa Millaa Falls, (e) Mulgrave River, (f) Wadda Creek.

Table 2 Major axes of interpopulation covariance matrices describing observed (**D**) and predicted (**B**) divergence due to sexual selection for male traits among six natural populations of guppies.

	D		B	
Trait	d_{max}	d_2	b_{max}	b_2
% variation explained	67.67	27.53	56.70	33.44
Body area	−0.456	−0.068	0.493	0.044
Tail area	−0.426	−0.033	−0.427	−0.181
Black	0.424	0.167	−0.396	−0.248
Fuzzy black	−0.457	0.030	0.497	−0.064
Orange	−0.201	−0.642	−0.082	0.627
Iridescence	0.156	−0.673	−0.401	0.376
Sperm no.	0.397	−0.316	0.060	0.604

been observed were less colourful than males from sites where predators were not observed (see Fig. 2, Table 3). This effect remained significant even after controlling for multiple tests (Table 3). Predation regime, however, did not influence the other male traits measured, nor sexual selection acting on any of these (multivariate $F_{1,4} = 0.176$, $P = 0.924$).

The role of genetic and geographical distance in determining population variation in male traits and sexual selection

Population differences in male traits were associated with genetic distances (Mantel test, $r = 0.624$, $P = 0.012$), but not linear geographical distances (Mantel test, $r = -0.063$, $P = 0.476$). In contrast, differences between populations in sexual selection were not correlated with either genetic distance (Mantel test, $r = -0.122$, $P = 0.621$) or geographical distance (Mantel test, $r = -0.068$, $P = 0.519$).

Discussion

Guppies from Trinidad, where they occur naturally, provide the most widely cited support for a correspondence between male trait expression and female mating preferences among populations (Endler, 1982; Houde &

Table 3 The effects of predation regime on male traits (pooled to population means) and sexual selection gradients (β) acting on these traits. Both original and corrected *P*-values are shown. ($p_{(FDR)}$) were calculated using the false discovery rate method to correct for multiple comparisons (Benjamini & Hochberg, 1995).

	Term	$F_{1,4}$	*P*	$p_{(FDR)}$
Mean male traits	Body	0.879	0.402	0.697
	Tail	0.848	0.409	0.697
	Black	1.001	0.374	0.697
	Fuzzy	0.049	0.835	0.868
	Orange	56.682	**0.002**	**0.028**
	Iridescence	8.981	**0.040**	0.280
	Sperm	0.223	0.662	0.800

Significant terms are shown in boldface.

Endler, 1990; Endler, 1995; but see Houde & Hankes, 1997; Schwartz & Hendry, 2007). Here, we found evidence for a match between male traits and the strength of sexual selection across six introduced Australian guppy populations: more than 50% of male trait variation among populations was due to the variation in sexual selection. This is high compared to the results of a comparable analysis, looking at population divergence in cuticular hydrocarbons of *Drosophila serrata*, which found that only 10% of male trait divergence could be attributed to divergent sexual selection alone (Chenoweth *et al.*, 2010). In *D. serrata*, male trait divergence was highly influenced by the genetic variance–covariance structure, indicating that genetic constraints played a large role. The pattern of multivariate genetic variation in a population strongly influences the trajectory along which each trait evolves (Schluter, 1996; Blows & Hoffmann, 2005), and constrains their evolution. In the guppy populations investigated here, there was considerable variation in the degree of alignment between the direction of sexual selection and that of male trait divergence within populations (ranging between 51° and 85°), suggesting that the potential for evolutionary response in the direction of selection is likely to vary across populations. Genetic constraints are one possible explanation for these results, but further investigation within a quantitative genetic framework would be needed to examine the nature of constraints on male trait adaptation across populations (Blows & Hoffmann, 2005; Blows, 2007).

Most studies relating male trait variation to sexual selection use measures of female preferences. In contrast to this, we estimated sexual selection gradients using paternity data. This provides an overall estimate of sexual selection which incorporates not only female precopulatory choice, but also post-copulatory processes such as female cryptic choice and sperm competition. Such post-copulatory processes have been shown to be important in driving the evolution of male sexual traits in the Alligator Creek population (Evans, 2010). We have shown previously that selection on male attractiveness and female preferences (Brooks & Endler, 2001a; Hall *et al.*, 2004) in the Alligator Creek population is unable to effect appreciable evolutionary change due to multivariate genetic constraint. In our study, Alligator Creek has the best alignment between male traits and sexual selection of all the populations we studied. Thus, the role of genetic architecture in constraining the response of male traits to sexual selection arising from precopulatory choice and post-copulatory processes within populations is likely to be widespread, with the constraints present in other populations investigated here being at least as large as those in Alligator Creek.

Rather than concluding that male trait divergence is due to one process (sexual selection, predator-induced selection or drift), we find evidence that all of them have influenced the observed pattern and that multivariate genetic constraints have also shaped the outcome. The weak but still important fit between sexual selection gradients and male trait divergence may be partially explained by natural selection. Predation has been previously shown to be the most important ecological factor influencing the evolution of male coloration (Millar *et al.*, 2006). In the present study, we also found that divergence in male ornamental traits was associated with differences in the presence/absence of piscivorous predators. However, historical selection regimes in the native source populations may have also played a role in shaping the constraints on the evolution of male traits and female preferences. Although the number of populations we studied was modest, the fact that visual-hunting piscivores appear to reduce both the proportion of males in the population (Head, 2005) and the level of orange coloration provides an interesting parallel with natural and introduced guppy populations within Trinidad. Orange coloration is one of the most consistently implicated cues of mate choice in guppies (Endler & Houde, 1995; Houde, 1997), including Australian populations (Brooks & Endler, 2001a; Blows *et al.*, 2003). Female preferences have been shown to co-evolve more slowly than male ornaments in guppies (Easty *et al.*, 2011) and are known to be highly variable (Zajitschek & Brooks, 2008; Easty *et al.*, 2011), rendering predictions about fine-scaled direction of sexual selection difficult. In addition, female-biased primary sex ratios and reduced courtship and harassment of females by males in the high-predation localities suggest that the relationship between sexual selection and predator-induced selection on male colour patterns may be complex (Head, 2005).

Phenotypic divergence was also correlated with genetic distance measured by neutral markers. It is unclear to what extent genetic distances reflect founder effects, as previous mtDNA analysis from the six populations investigated here point to gene flow or to two female source populations in Guyana and Trinidad (Lindholm *et al.*, 2005). The role of common female

founders in explaining trait divergence may be modest, due to the fact that the populations of Alligator Creek and Mena Creek share a single mtDNA type, but did not show similarity in sexual selection. Male-biased gene flow between introduced guppy populations is also likely to have occurred (Lindholm *et al.*, 2005), but the effects of gene flow and admixture of founder populations cannot be fully disentangled.

While some Trinidad populations have been separated for 200 000 years (Fajen & Breden, 1992), the North Queensland populations have only been introduced in the last century. Despite this short time frame, and bottlenecks which have reduced genetic diversity (Lindholm *et al.*, 2005), sexual selection differs substantially between the populations in ways that have shaped male sexual trait variation. Theoretic models of mate choice evolution show, however, that in the vast majority of circumstances, direct selection on choice and signal will swamp the indirect co-evolutionary processes that cause an association between trait and preference (Kokko *et al.*, 2006). Direct selection on male ornamentation in a new environment is likely to initially involve direct adaptation to the signalling environment including signal propagation considerations and the presence of predators (Endler, 1987). Likewise, direct selection on choice might also be shaped more by factors such as predators and food in a new environment than by the more subtle effects of signaller–receiver co-evolution. Further, in guppies, heritabilities of male traits are much higher than heritabilities of female choice (Houde, 1992; Brooks & Endler, 2001a,b; Hall *et al.*, 2004; Hughes *et al.*, 2005), suggesting greater potential for male traits to respond rapidly during introduction to new environments.

Conclusions

Here, we show that populations of recently introduced guppy populations differ significantly in both male sexual traits and sexual selection on these traits. Furthermore, we show the existence of substantial among-population covariation between sexual selection and male traits. We thus demonstrate that differences in sexual selection between populations are an important driver of population variation in male traits, despite the effects of other factors (e.g. ecological selection, evolutionary history) that are expected to constrain evolutionary responses. Our results may have important implications for understanding how sexual selection contributes to population divergence and speciation. In addition, the rapid divergence in male traits under different ecological conditions highlights how introduced species are likely to adapt to new environments. Further studies determining the generality of our results in other systems, as well as studies that incorporate quantitative genetic breeding designs, will be important next steps for research on how organisms adapt to new environments and how sexual selection contributes to trait divergence between the populations.

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References

Arnold, S.J., Pfrender, M.E. & Jones, A.G. 2001. The adaptive landscape as a conceptual bridge between micro- and macro-evolution. *Genetica* **112–113**: 9–32.

Becher, S.A. & Magurran, A.E. 2004. Multiple mating and reproductive skew in Trinidadian guppies. *Proc. Roy. Soc. B.* **271**: 1009–1014.

Becher, S.A., Russell, S.T. & Magurran, A.E. 2002. Isolation and characterization of polymorphic microsatellites in the Trinidadian guppy (*Poecilia reticulata*). *Mol. Ecol. Notes* **2**: 456–458.

Benjamini, Y. & Hochberg, Y. 1995. Controlling the false discovery rate - a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B. Stat. Methodol.* **57**: 289–300.

Blows, M.W. 2007. A tale of two matrices: multivariate approaches in evolutionary biology. *J. Evol. Biol.* **20**: 1–8.

Blows, M.W. & Hoffmann, A.A. 2005. A reassessment of genetic limits to evolutionary change. *Ecology* **86**: 1371–1384.

Blows, M.W., Brooks, R. & Kraft, P.G. 2003. Exploring complex fitness surfaces: multiple ornamentation and polymorphism in male guppies. *Evolution* **57**: 1622–1630.

Boughman, J.W. 2001. Divergent sexual selection enhances reproductive isolation in sticklebacks. *Nature* **411**: 944–947.

Boul, K.E., Funk, W.C., Darst, C.R., Cannatella, D.C. & Ryan, M.J. 2007. Sexual selection drives speciation in an Amazonian frog. *Proc. Roy. Soc. B.* **274**: 399–406.

Bowerman, B.L. & O'Connell, R.T. 1990. *Linear Statistical Models: An Applied Approach*, Duxbury Press, Belmont, CA.

Brooks, R. & Endler, J.A. 2001a. Direct and indirect sexual selection and quantitative genetics of male traits in guppies (*Poecilia reticulata*). *Evolution* **55**: 1002–1015.

Brooks, R. & Endler, J.A. 2001b. Female guppies agree to differ: phenotypic and genetic variation in mate-choice behaviour and the consequences for sexual selection. *Evolution* **55**: 1644–1655.

Chenoweth, S.F., Rundle, H.D. & Blows, M.W. 2010. The contribution of selection and genetic constraints to phenotypic divergence. *Am. Nat.* **175**: 186–196.

Claridge, M.F. & Morgan, J.C. 1993. Geographical variation in acoustic signals of the planthopper, Nilaparvata bakeri (Muir), in Asia: species recognition and sexual selection. *Biol. J. Linn. Soc.* **48**: 267–281.

Draper, N.R. & John, J.A. 1988. Response-surface designs for quantitative and qualitative variables. *Technometrics* **30**: 423–428.

Easty, L.K., Schwartz, A.K., Gordon, S.P. & Hendry, A.P. 2011. Does sexual selection evolve following introduction to new environments? *Anim. Behav.* **82**: 1085–1095.

Endler, J.A. 1980. Natural selection on color patterns in *Poecilia reticulata*. *Evolution* **34**: 76–91.
Endler, J.A. 1982. Convergent and divergent effects of natural selection on color patterns in two fish faunas. *Evolution* **36**: 178–188.
Endler, J.A. 1987. Predation, light intensity and courtship behaviour in *Poecilia reticulata* (Pisces: Poeciliidae). *Anim. Behav.* **35**: 1376–1385.
Endler, J.A. 1990. On the measurement and classification of colour in studies of animal colour patterns. *Biol. J. Linn. Soc.* **41**: 315–352.
Endler, J.A. 1995. Multiple-trait coevolution and environmental gradients in guppies. *Trends Ecol. Evol.* **10**: 22–29.
Endler, J.A. & Houde, A.E. 1995. Geographic variation in female preferences for male traits in *Poecilia reticulata*. *Evolution* **49**: 456–468.
Evans, J.P. 2010. Quantitative genetic evidence that males trade attractiveness for ejaculate quality in guppies. *Proc. Natl. Acad. Sci. B.* **277**: 3195–3201.
Fajen, A. & Breden, F. 1992. Mitochondrial DNA sequence variation among natural populations of the Trinidad guppy, *Poecilia reticulata*. *Evolution* **46**: 1457–1465.
Geffen, E.L.I., Anderson, M.J. & Wayne, R.K. 2004. Climate and habitat barriers to dispersal in the highly mobile grey wolf. *Mol. Ecol.* **13**: 2481–2490.
Godin, J.-G.J. & McDonough, H.E. 2003. Predator preference for brightly colored males in the guppy: a viability cost for a sexually selected trait. *Behav. Ecol.* **14**: 194–200.
Gordon, S.P., Reznick, D.N., Kinnison, M.T., Bryant, M.J., Weese, D.J., Rasanen, K. *et al.* 2009. Adaptive changes in life history and survival following a new guppy introduction. *Am. Nat.* **174**: 34–45.
Hall, M., Lindholm, A.K. & Brooks, R. 2004. Direct selection on male attractiveness and female preference fails to produce a response. *BMC Evol. Biol.* **4**: L1. http://www.biomedcentral.com/1471-2148/4/1.
Harvey, P.H. & Pagel, M.D. 1991. *The Comparative Method in Evolutionary Biology*. Oxford University Press, Oxford.
Head, M.L. 2005. *Evolutionary Consequences of the Costs of Mate Choice. Ph.D*. The University of New South Wales, Sydney.
Hendry, A.P., Farrugia, T.J. & Kinnison, M.T. 2008. Human influences on rates of phenotypic change in wild animal populations. *Mol. Ecol.* **17**: 20–29.
Hill, G.E. 1994. Geographic variation in male ornamentation and female mate preference in the house finch: a comparative test of models of sexual selection. *Behav. Ecol.* **5**: 64–73.
Hine, E., McGuigan, K. & Blows, M.W. 2011. Natural selection stops the evolution of male attractiveness. *Proc. Natl. Acad. Sci. USA* **108**: 3659–3664.
Hoekstra, H.E., Hoekstra, J.M., Berrigan, D., Vignieri, S.N., Hoang, A., Hill, C.E. *et al.* 2002. Strength and tempo of directional selection in the wild. *Proc. Natl. Acad. Sci. USA* **98**: 9157–9160.
Houde, A.E. 1992. Sex-linked heritability of a sexually selected character in a natural population of *Poecilia reticulata* (Pisces: Poeciliidae) (guppies). *Heredity* **69**: 229–235.
Houde, A.E. 1993. Evolution by sexual selection: what can population comparisons tell us? *Am. Nat.* **141**: 796–803.
Houde, A.E. 1997. *Sex, Color and Mate Choice in Guppies*. Princeton University Press, Princeton, NJ.
Houde, A.E. & Endler, J.A. 1990. Correlated evolution of female mating preferences and male color patterns in the guppy *Poecilia reticulata*". *Science* **248**: 1405–1408.
Houde, A.E. & Hankes, M.A. 1997. Evolutionary mismatch of mating preferences and male color patterns in guppies. *Anim. Behav.* **53**: 343–351.
Hughes, K.A., Rodd, F.H. & Reznick, D.N. 2005. Genetic and environmental effects on secondary sex traits in guppies (*Poecilia reticulata*). *J. Evol. Biol.* **18**: 35–45.
Johnston, R.F. & Selander, R.K. 1973. Evolution in the house sparrow. III. variation in size and sexual dimorphism in Europe and North and South America. *Am. Nat.* **107**: 373–390.
Kingsolver, J.G. & Pfennig, D.W. 2007. Patterns and power of phenotypic selection in nature. *Bioscience* **57**: 561–572.
Kingsolver, J.G., Hoekstra, H.E., Hoekstra, J.M., Berrigan, D., Vignieri, S.N., Hill, C.E. *et al.* 2001. The strength of phenotypic selection in natural populations. *Am. Nat.* **157**: 245–261.
Kokko, H., Jennions, M.D. & Brooks, R. 2006. Unifying and testing models of sexual selection. *Annu. Rev. Ecol. Evol. Syst.* **37**: 43–66.
Kolbe, J.J., Glor, R.E., Schettino, L.R., Lara, A.C., Larson, A. & Losos, J.B. 2004. Genetic variation increases during biological invasion by a Cuban lizard. *Nature* **431**: 177–181.
Krzanowski, W.J. 1979. Between-groups comparison of principal components. *J. Am. Stat. Assoc.* **74**: 703–707.
Lande, R. & Arnold, S.J. 1983. The measurement of selection on correlated characters. *Evolution* **37**: 1210–1226.
Lindholm, A.K., Breden, F., Alexander, H.J., Chan, W.K., Thakurta, S.G. & Brooks, R. 2005. Invasion success and genetic diversity of introduced populations of guppies *Poecilia reticulata* in Australia. *Mol. Ecol.* **14**: 3671–3682.
Losos, J.B., Warheit, K.I. & Schoener, T.W. 1997. Adaptive differentiation following experimental island colonization in Anolis lizards. *Nature* **387**: 70–73.
Magnhagen, C. 1991. Predation risk as a cost of reproduction. *Trends Ecol. Evol.* **6**: 183–186.
Marshall, T.C., Slate, J., Kruuk, L.E.B. & Pemberton, J.M. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Mol. Ecol.* **7**: 639–655.
Mathews, I.M., Evans, J.P. & Magurran, A.E. 1997. Male display rate reveals ejaculate characteristics in the Trinidadian guppy *Poecilia reticulata*. *Proc. Roy. Soc. B.* **264**: 695–700.
Millar, N.P., Reznick, D.N., Kinnison, M.T. & Hendry, A.P. 2006. Disentangling the selective factors that act on male colour in wild guppies. *Oikos* **113**: 1–12.
Peakall, R. & Smouse, P.E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes* **6**: 288–295.
Pusey, B., Kennard, M. & Arthington, A. 2004. *Freshwater Fishes of North-Eastern Australia*. CSIRO Publishing, Melbourne.
R Development Core Team 2012. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
Ramachandran, S., Deshpande, O., Roseman, C.C., Rosenberg, N.A., Feldman, M.W. & Cavalli-Sforza, L.L. 2005. Support from the relationship of genetic and geographic distance in human populations for a serial founder effect originating in Africa. *Proc. Natl. Acad. Sci. USA* **102**: 15942–15947.
Reznick, D.N. & Ghalambor, C.K. 2001. The population ecology of contemporary adaptations: what empirical studies

reveal about the conditions that promote adaptive evolution. *Genetica* **112**: 183–198.
Reznick, D., Bryga, H. & Endler, J.A. 1990. Experimentally induced life-history evolution in a natural population. *Nature* **346**: 357–359.
Reznick, D.N., Shaw, F.H., Rodd, F.H. & Shaw, R.G. 1997. Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). *Science* **275**: 1934.
Ritchie, M.G. 1991. Female preference for "song races" of *Ephippiger ephippiger* (Orthoptera: Tettigoniidae). *Anim. Behav.* **42**: 518–520.
Ruell, E.W., Handelsman, C.A., Hawkins, C.L., Sofaer, H.R., Ghalambor, C.K. & Angeloni, L. 2013. Fear, food and sexual ornamentation: plasticity of colour development in Trinidadian guppies. *Proc. Roy. Soc. B.* **280**: 1758.
Rundle, H.D., Chenoweth, S.F., Doughty, P. & Blows, M.W. 2005. Divergent selection and the evolution of signal traits and mating preferences. *PLoS Biol.* **3**: e368.
Schluter, D. 1996. Adaptive radiation along genetic lines of least resistance. *Evolution* **50**: 1766–1774.
Schluter, D. 2000. *The Ecology of Adaptive Radiation*. Oxford University Press, Oxford.
Schwartz, A.K. & Hendry, A.P. 2007. A test for the parallel co-evolution of male colour and female preference in Trinidadian guppies (*Poecilia reticulata*). *Evol. Ecol. Res.* **9**: 71–90.
Seckinger, J., Brinkman, H. & Meyer, A. 2002. Microsatellites in the genus *Xiphophorus*, developed in *Xiphophorus montezumae*. *Mol. Ecol. Notes* **2**: 4–6.
Sokal, R.R. & Rohlf, F.J. 1995. *Biometry: The Principles and Practice of Statistics in Biological Research*. W.H. Freeman, New York, NY.
Svensson, E.I. & Gosden, T.P. 2007. Contemporary evolution of secondary sexual traits in the wild. *Funct. Ecol.* **21**: 422–433.
Taylor, J. 1999. *The Evolution of Repetitive DNA in the Guppy (Poecilia reticulata)* and the Genetic Structure of Natural Guppy Populations. Ph.D. Simon Fraser University, Burnaby, BC.
Verrell, P.A. 1999. Geographic variation in sexual behaviour: sex, signals and speciation. In: *Geographic Variation in Behaviour* (S.A. Foster & J.A. Endler, eds), pp. 262–286. Oxford University Press, New York, NY.
Weese, D.J., Gordon, S.P., Hendry, A.P. & Kinnison, M.T. 2010. Spatiotemporal variation in linear selection on body color in wild guppies (*Poecilia reticulata*). *Evolution* **64**: 1802–1815.
Zajitschek, S.R.K. & Brooks, R.C. 2008. Distinguishing the effects of familiarity, relatedness, and color pattern rarity on attractiveness and measuring their effects on sexual selection in guppies (*Poecilia reticulata*). *Am. Nat.* **172**: 843–854.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Linear selection gradients for each male trait in the feral guppy populations in North Queensland: Alligator Creek ('Ack'), Big Crystal Creek ('Crc'), Mena Creek ('Mnc'), Millaa Millaa Falls ('Mlm'), Mulgrave River ('Ulg'), Wadda Creek ('Wdd').

Table S1 Interpopulation variance–covariance matrix among-population means (**D**) for the seven male traits for the six natural populations of *Poecilia reticulata*.

Table S2 Interpopulation variance–covariance matrix of directional sexual selection gradients (**B**) acting on the seven male traits for the six natural populations of *Poecilia reticulata*.

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Figure S1: Linear selection gradients for each male trait in the feral guppy populations in North Queensland: Alligator Creek (“Ack), Big Crystal Creek (“Crc”), Mena Creek (“Mnc”), Millaa Millaa Falls (“Mlm”), Mulgrave River (“Ulg”), Wadda Creek (“Wdd”).

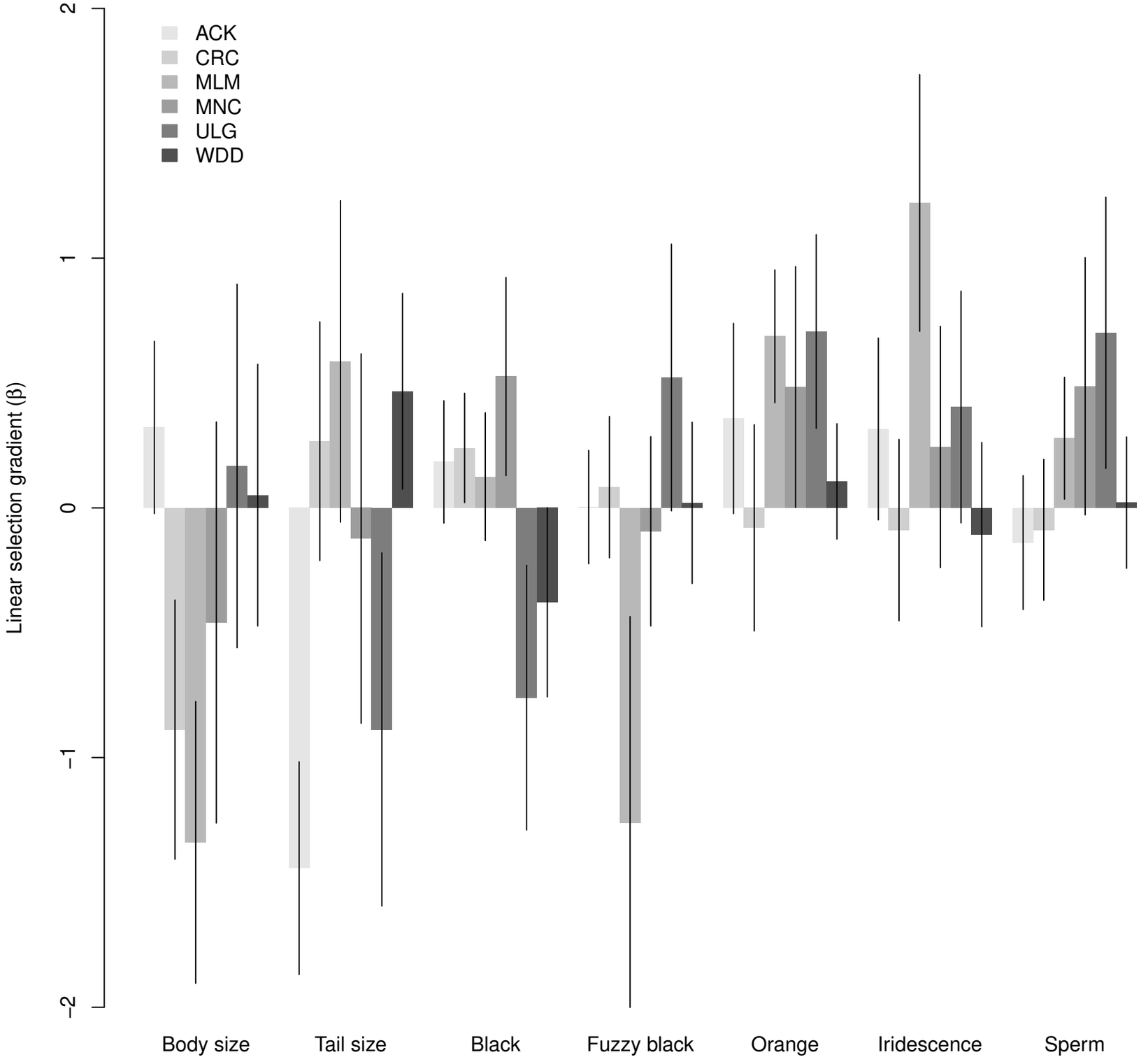
ACK
CRC
MLM
MNC
ULG
WDD
Linear selection gradient (β)
2
1
0
−1
−2
Body size
Tail size
Black
Fuzzy black
Orange
Iridescence
Sperm

Table S1. Inter-population variance-covariance matrix among-population means (**D**) for the seven male traits for the six natural populations of *Poecilia reticulata*

	Body	Tail	Black	Fuzzy	Orange	Iridescence	Sperm
Body	0.168						
Tail	0.178	0.304					
Black	-0.074	-0.037	0.050				
Fuzzy	0.046	0.041	-0.021	0.015			
Orange	0.055	0.071	-0.031	0.005	0.121		
Iridescence	0.001	-0.003	-0.006	-0.003	0.035	0.013	
Sperm	-0.057	-0.037	0.033	-0.023	0.045	0.026	0.128

Table S2. Inter-population variance-covariance matrix of directional sexual selection gradients (**B**) acting on the seven male traits for the six natural populations of *Poecilia reticulata*

	Body	Tail	Black	Fuzzy	Orange	Iridescence	Sperm
Body	0.440						
Tail	-0.395	0.661					
Black	-0.147	0.059	0.221				
Fuzzy	0.302	-0.260	-0.119	0.357			
Orange	-0.006	-0.069	-0.033	-0.054	0.100		
Iridescence	-0.162	0.035	0.014	-0.224	0.120	0.235	
Sperm	0.001	-0.018	-0.057	0.023	0.081	0.057	0.114

Chapter 8

Environmental variation and the maintenance of polymorphism: The effect of ambient light spectrum on mating behaviour and sexual selection in guppies

Gamble, S, Lindholm, AK, Endler, JA & Brooks, R. 2003. Environmental variation and the maintenance of polymorphism: The effect of ambient light spectrum on mating behaviour and sexual selection in guppies. *Ecology Letters* 6: 463-472.

Ecology Letters, (2003) **6**: 463–472

REPORT

Environmental variation and the maintenance of polymorphism: the effect of ambient light spectrum on mating behaviour and sexual selection in guppies

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Abstract

The intensity of sexual selection is influenced by environmental conditions because these conditions influence signal propagation and the risks of the signal being exploited by predators and parasites. We explore the possibility that spatial or temporal heterogeneity in environmental signalling conditions (in this case light spectrum) may induce fluctuating sexual selection on male behaviour and ornamentation in guppies. We used shade cloth and filters to experimentally manipulate light spectrum, mimicking conditions found naturally: early morning/late afternoon light (SC treatment), midday forest shade (F89 filter treatment) and midday woodland shade (F55 filter treatment). Females were more responsive to male courtship and males were less likely to attempt sneak copulations under F55 light than the other two treatments. By contrast, male display rate was not influenced by treatment. Females tended to prefer the same males under SC and F55 light, but attractiveness in these treatments was unrelated to attractiveness under F89 light. There were similarities among treatments in the traits that females preferred: females preferred males with larger areas of orange in all three treatments. There were, however, also some differences, including preference for larger males under F89 light and for smaller males under the other treatments. Overall, the influence of ambient light spectrum on the relative importance of mate choice and male sneak copulation may have important implications for the mode and strength of sexual selection in different environments. The findings on attractiveness and preference functions, however, suggest that light spectrum only weakly affects the direction of sexual selection by female choice.

Keywords

Polymorphism, mate choice, lek paradox, sneak copulation, *Poecilia reticulata*.

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INTRODUCTION

One of the most important challenges in evolutionary biology is to understand the maintenance of additive genetic variation in traits that influence fitness (Barton & Turelli 1989). This is particularly relevant to the evolution of mate choice, because there must be substantial variation in male genetic quality – as signalled by displays and ornaments – in order for choosy females to derive any indirect (genetic) benefits over females that are not choosy (Kirkpatrick & Ryan 1991; Andersson 1994). Considerable attention has been devoted to this issue (Andersson 1994; Pomiankowski & Møller 1995; Rowe & Houle 1996), particularly because the concomitant action of natural selection on 'quality' and sexual selection on 'attractiveness' is generally expected to erode genetic variation by fixing favourable alleles (Taylor & Williams 1982; Kirkpatrick & Ryan 1991; Andersson 1994).

Male guppies display one of the most prodigious examples of polymorphism: there are large differences among males within populations in the size, number and position of black, fuzzy black, orange and various iridescent spots, and in body and tail size. Moreover, there are differences among males in the frequency of sigmoid courtship displays and attempted sneak copulations. All of these traits are important determinants of male mating success, and thus are expected to be under directional sexual selection (Houde 1987, 1997; Endler & Houde 1995; Brooks & Endler 2001). Here, we test the importance of one

possible process that may maintain variation in male colour patterns and mating strategies in the guppy (*Poecilia reticulata*): fluctuating sexual selection due to temporal and spatial variation in the ambient light (irradiance) spectrum.

In the wild, male guppies tend to court most ardently early in the morning and late in the afternoon when incident light intensity is low and purplish in colour. By contrast, courtship is less intense and male attempts at sneaky mating relatively more frequent in the middle of the day when light intensity is higher and closer to a greenish (in forest shade), bluish (woodland shade), or other diurnal spectrum (Endler 1987, 1993a; Long & Rosenqvist 1998). These differences may be due to the effects that light intensity and spectrum have on how the colourful mate-attractant patterns of males are perceived by females and by predators. Under early/late conditions, colour patterns are more conspicuous to female guppies and less so to their visually hunting predators (Endler 1991, 1993a).

Evidence that the outcome of sexual selection is influenced by local differences in predation (Endler 1987, 1991; Endler & Houde 1995) or the environmental properties that effect signal propagation (Ryan & Wilczynski 1991; Endler 1992, 1993a,b, 2000; Boughman 2001) might profitably be extrapolated to help explain within-population variation in mating tactics and sexual displays. That is, environmental variation may cause fluctuations in the net strength and direction of selection and thus different phenotypes may be favoured at different times or places. Here, we test experimentally the possibility that one component of environmental variation in incident lighting – variation in ambient light spectrum – is responsible for variation in male and female mating strategies and in male ornamentation in guppies.

METHODS

All guppies used for this study were second-generation laboratory-bred individuals descended from individuals collected in 1999 from Alligator Creek, Queensland, Australia (Brooks & Endler 2001). Guppies from this location are comparable in coloration and mating preference to populations found in low to medium predation sites in Trinidad (Brooks & Endler 2001). Sixty sexually mature males were drawn from a stock population and photographed under ice anaesthesia, using a digital camera, on white background with a ruler included in the photograph for calibration. Male colour patterns, and tail and body outlines were traced from each digital image using Measure Master 3.44 (Leading Edge Corporation, Adelaide, Australia).

The ambient light treatments

Light from one fluorescent tube (Narma lumofluor 36W) was filtered through coloured cellophane theatrical Light Filters or shade cloth to yield each of three treatments, F55, F89 and SC (Fig. 1). We used Roscolux filter numbers 55 ('Lilac') and 89 ('Green') for filter treatments F55 and F89, respectively, and the third treatment (SC) used only shade cloth. We kept the light intensity relatively low and minimized differences in light intensity between the treatments by varying the numbers of layers of filter or shade cloth. The mean ± SD total irradiances (400–700 nm, Skye PAR meter) at six standard positions at water surface for each treatment were (in $\mu mol\ m^{-2}\ s^{-1}$): F55, 6.43 ± 0.83; F89, 5.53 ± 1.60; and SC, 5.57 ± 1.65. The lamps emit little UV, so the total irradiances over the entire guppy visible range (300–700 nm) are very similar, 6.51,

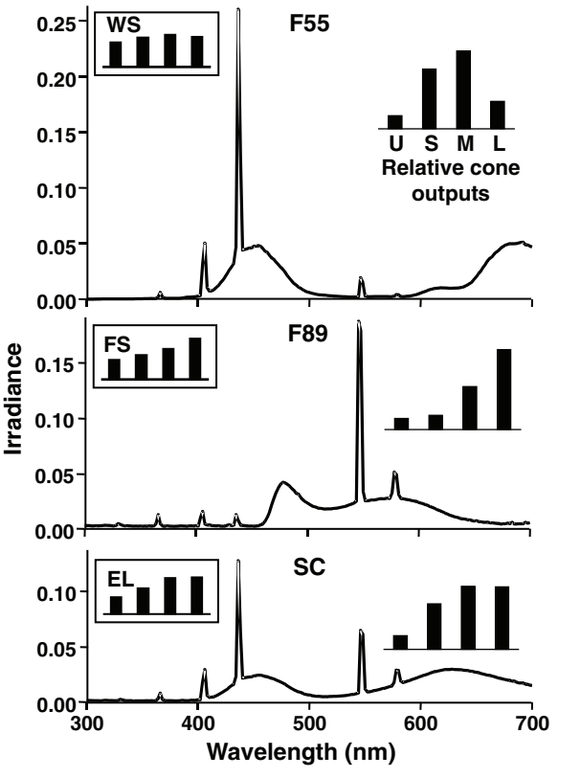

Figure 1 Irradiance spectra of the three ambient light treatments (F55, SC and F89) and their relative cone stimulation of guppy cones. Irradiance spectra were measured at water level; units are: $\mu mol\ m^{-2}\ s^{-1}\ nm^{-1}$. Estimated relative cone stimulations (Endler 1991) by these light environments are shown in the upper right corner of each spectrum: U, UV-sensitive cone; S, short-wavelength-sensitive cone; M, medium-wavelength-sensitive cone; L, long-wavelength-sensitive cone. Estimated relative cone stimulations of similar natural light environments are shown in the upper left corner of each spectrum (same cone order): WS, woodland shade; EL, early/late; FS, forest shade (for natural ambient light spectra see Endler 1993a). The L cone responds little to the longest wavelengths, which is why the part of the spectrum above about 650 nm has little effect on the relative cone outputs.

5.84, and 5.72 μmol m^{-2} s^{-1}, respectively. We minimized light intensity differences because they are known to affect courtship behaviour and mate choice (Endler 1987; Long & Rosenqvist 1998; Reynolds *et al.* 1993). These light intensities are similar to those experienced by guppies in natural habitats (Endler 1987, 1991, 1993a).

The treatments were chosen for spectral similarity to natural environments (Endler 1993a). The light from fluorescent lamps contains peaks and has a different shape from that of natural environments (Fig. 1); however, what counts is how the four guppy cones are stimulated (Endler 1991; Fleishman & Endler 2000). For example, the emission spectra of some of the phosphors on computer monitors also contains peaks, and the 'white' produced by a computer monitor is not spectrally flat (true white) but is as variable as Fig. 1. The phosphors in monitors were designed to stimulate the three human cones and because they are stimulated in the same way as in natural scenes, colour images look natural on computers (and in digital cameras and photographs). We are taking advantage of the same phenomenon in guppies by choosing irradiance spectra that stimulate guppy cones in ways similar to the natural environments (Fig. 1). Note that because guppies have four cones (compared to humans' three) and cones that absorb in different wavelength ranges than humans, what we would call similar is not what guppies would call similar, and vice versa (detailed discussion in Fleishman & Endler 2000). Cone stimulation ratios were calculated as in Endler (1991). The F55 treatment is similar to Woodland Shade environments (WS in Fig. 1) in that it stimulates the S and M cones the most and the other two less so. The F89 treatment stimulates the L cones the most, and shows declining stimulation for the other cones, and is similar to Forest Shade (FS in Fig. 1). The SC treatment stimulates the M and L cones roughly equally, and less so the S and U cones and thus resembles the Early/Late environment (EL in Fig. 1). Because the differences are greater than nature in F55 and F89, these two environments are more chromatic (more 'saturated' in human terms) than the similar natural environments, but not nearly as chromatic as the very strongly coloured lights used in experiments by Long & Houde (1989) and Brooks & Caithness (1995b). The colours ('hues' in human terms) are similar to that of nature because the relative stimulations of the four cones are the same qualitatively (same rank order of stimulation intensity). For this reason, we cannot distinguish differences in behaviour associated with hue from those associated with chroma (if guppies have similar perceptual axes), but because the treatments are matched for intensity, we will be able to tell if some aspect of colour (spectral shape; 'hue' or 'chroma' or both) is important to courtship behaviour.

Behaviour tanks (volume 130 l) were set up in a constant temperature room (26°C). Each tank was in an individual enclosure sealed from light contamination with black felt cloth. The room in which the tanks and enclosures were held was light-proof, and the light was switched off during feeding and observations. Thus, all incident light in the tank came through the coloured filter/shade cloth.

Behaviour observations

Males were randomly divided into six groups of 10 males for behaviour trials. The morning before a trial began, a group of 10 males were photographed and 10 females were caught from a virgin stock and introduced to a behaviour tank. The males were added 8 h later, and behaviour observations were conducted the next two mornings between 0700 and 0900 hours, before the fish were fed. Fish were left in the tank for 2 weeks, after which all fish were removed, the water was partially changed, and the screens were changed. Ten new virgin females were put in the tank and the males reintroduced to the tank after 8 h. This was repeated 2 weeks later such that each set of 10 males underwent all three treatments. Each of the six possible sequences of treatment order was used, on a different group of males. There are, thus, six replicate applications of each treatment, and 10 males nested within each replicate. The behaviour of individual females within a replicate could not be distinguished as females cannot be recognized individually. We thus interpret our results as six independent replicate measures per treatment of how populations of females respond to males under the spectrum of incident light presented. We are interested in male fitness under different conditions rather than individual variation in female choice, so pooling females within trials does not result in loss of relevant information.

In each observation session, each male was observed (individuals could be identified from photographs) for two 5-min periods (total 100 min of observation per tank per day). The observer (S.G.) observed males in different random order for the first and second set of observations. Male behaviour and the female response to male courtship were scored using standard methods (Houde 1987, 1997; Endler & Houde 1995). We were particularly interested in the numbers of sneak copulations a male attempted, the number of sigmoid displays he executed and the proportion of these displays that elicited a female 'glide' response, or a more positive (circling or mating) response (see Houde 1997 for a full explanation of female response behaviour).

Statistical analysis

We performed all analyses on SPSS V11.0 software. We used the Lilliefors test for normality and transformed measures where appropriate to satisfy assumptions of normality.

Because each male was subjected to all three light colour treatments, we used repeated-measures analysis of variance to test for the effects of treatment on the group of 10 females involved in a specific trial and the group of 10 males that underwent the three treatments together. These effects are given, respectively, by within-subjects contrasts for treatment and treatment by group interaction and by the between-subjects contrast for group. Separate analyses were performed for female responsiveness, male display rate and male gonopodial thrust rate. Display rate did not satisfy the assumption of sphericity (multivariate normality and lack of correlation among variables), and so Greenhouse–Geisser approximations (Quinn & Keough 2002) were used to arrive at appropriate degrees of freedom. Female responsiveness and male gonopodial thrust rate satisfied sphericity assumptions. Because the group of females is the appropriate unit of replication for tests of treatment effects, we adjusted the denominator degrees of freedom accordingly.

To explore repeatability of male attractiveness and correlations between male attractiveness measures in the three treatments, we first removed the effect of variation among trials in female responsiveness by standardizing male attractiveness scores within trials. We then estimated repeatability from variance components extracted from a one-way analysis of variance after the methodology given by Becker (1992). We estimated Pearson's product–moment correlation coefficients to compare the attractiveness of males under each pair of treatments.

We used the same standardized data to test for differences in preference slopes among treatments using nested analysis of covariance (after Brooks & Endler 2001). To do this we tested the significance of treatment, group of females within treatment, the trait concerned (covariate), the treatment by covariate and covariate by group within treatment interactions. We do not report the treatment or group within treatment significance tests because standardization removed any differences among groups and among treatments. The interaction terms test for significant differences among treatments and among groups nested within treatment in slope of preference for the trait. Only if these interaction terms are not significant can the covariate term be interpreted. The covariate term tests the significance of the common regression slope, in biological terms the significance of the shared preference shown by females from all treatments.

We built multiple regression models of the traits related to attractiveness under each treatment by choosing the model that minimized Mallows' *Cp* statistic relative to the number of parameters in the model, an approach that identifies the model that maximizes explanatory power without over-fitting (Draper & Smith 1981).

RESULTS

Female responsiveness and the frequency of sneak copulation attempts by males differed significantly among treatments (Fig. 2, Table 1). Under F55 light, females responded positively to males more often than under F89 or SC light (least significant difference, F55 – F89, $P = 0.001$; F55 – SC, $P = 0.005$; F89 – SC, $P = 0.314$). Under F55 light males attempted fewer sneak copulations than under F89 or SC light (LSD, F55 – F89, $P = 0.000$; F55 – SC, $P = 0.000$; F89 – SC, $P = 0.401$). There were no significant differences in male display rate among treatments (Fig. 2, Table 1), consistent with our equalizing the light intensities among treatments.

The attractiveness of individual males and their tendency to attempt sneak copulations were not significantly repeatable across treatments, indicating that either behaviour is

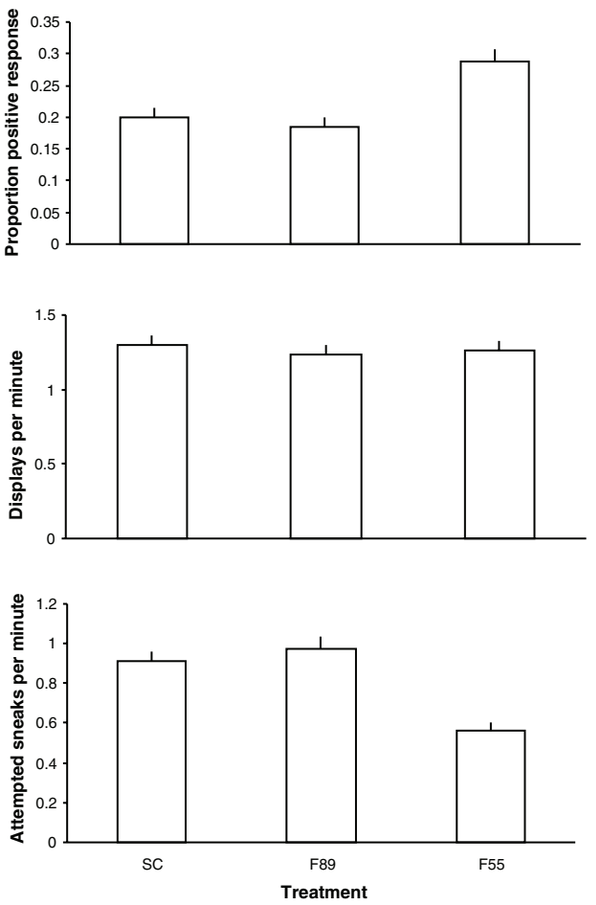

Figure 2 Means and standard errors of proportion of male displays receiving a positive response from females, male display rate and attempted sneak (gonopodial thrust) rate under the three treatments.

Table 1 Repeated-measures analysis of variance of the effect of light spectrum treatment, group of females and group of males on female responsiveness, male display rate and male attempted sneak copulation rate

	Within-subject contrasts			Between-subjects effects		
	d.f.	*F*	*P*	d.f.	*F*	*P*
Female response						
Treatment	2, 10	7.30	0.011			
Group of females	10, 104	2.65	0.006			
Group of males				5, 52	5.99	0.000
Male display rate						
Treatment	1.8, 9.0*	0.44	0.526			
Group of females	9.0, 93.4*	3.31	0.007			
Group of males				5, 52	1.63	0.169
Male attempted sneak rate						
Treatment	2, 10	19.80	0.003			
Group of females	10, 104	12.19	0.000			
Group of males				5, 52	5.84	0.000

*Greenhouse–Geisser approximation as sphericity could not be assumed.

related to light environment or too variable to show any pattern (Table 2). The low repeatability of male attractiveness is further illustrated by the low, non-significant correlations between male attractiveness under the SC spectrum and F89 and the F89 and F55 treatments, although male attractiveness under the SC and F55 treatments was significantly correlated (Fig. 3). The number of sigmoid displays performed by a male per 10-min sampling period was significantly repeatable across treatments (Table 2), again consistent with our equalizing the light intensities of the treatments. Larger males displayed less often than smaller males, and this relationship was consistent across treatments [ANCOVA: Body (covariate) $F_{1,174} = 7.55$, $P = 0.007$; Treatment $F_{2,174} = 1.17$, $P = 0.313$; Treatment × Body $F_{2,174} = 1.06$, $P = 0.350$]. Larger males also attempted sneak copulations significantly more often [ANCOVA: Body(covariate) $F_{1,174} = 10.66$, $P = 0.001$; Treatment $F_{2,174} = 2.67$, $P = 0.072$; Treatment × Body $F_{2,174} = 2.04$, $P = 0.133$).

The only trait for which there were significantly different preference slopes across treatments was male body size (Table 3, Fig. 4a). There was a significant positive preference slope for relative orange area common to the three treatments (Table 3, Fig. 4b). Linear preference functions for other traits were neither significantly different from one another, nor were the common slopes significant (Table 3).

Table 2 Estimated repeatability of male attractiveness to females, display rate and attempted sneak copulation rate

	r	SE	*P*
Attractiveness	0.104	0.08	0.087
Display rate	0.299	0.08	0.000
Attempted sneak rate	0.059	0.08	>0.2

Multiple regression models of male attractiveness for each of the three treatments included significant, positive partial contributions of orange coloration (Table 4). Tail area was included as a positive contributor to the models for both F89 and F55 treatments. Apart from these similarities, each model included at least one variable that was not included in the other models. Each of the three multiple regression models were significantly better than a null model at predicting male attractiveness.

DISCUSSION

Mating behaviour/strategy

Here, we provide the first experimental evidence that at controlled light intensity, female sexual responsiveness is affected by the ambient light spectrum. Moreover, the light spectra in the three treatments represent similar hues and saturations to the spectra in the natural environments in which guppies occur. Females are sexually more responsive under F55 light (strong S and M cone adaptation, as in woodland shade) than under F89 (strong L cone stimulation as in forest shade) or SC light (typical of normal maximum courtship times early and late in the day). There is a possible physiological explanation for this. The F55 irradiance strongly stimulates the S and M cones (Fig. 1). This means that under this irradiance, guppies will have less sensitive S and M cones, and more sensitive U and L cones (Endler 1991; Vorobyev & Osorio 1998). The common orange-, UV- and violet-reflecting spots will show higher visual contrast because they will produce light that guppy eyes are particularly sensitive to in this light environment, and the effect will be much stronger than in nature where natural irradiance does not have such a strong differential cone stimulation. These more contrasting patterns may, therefore,

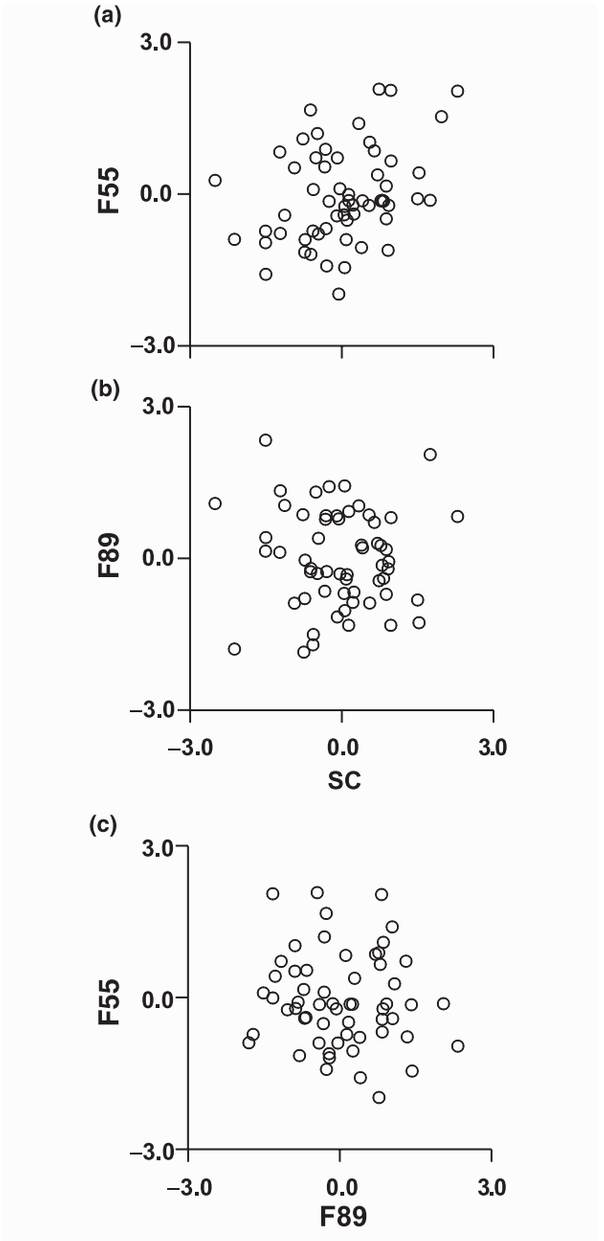

Figure 3 Correlations between the attractiveness of individual males in each pairwise combination of the three treatments: (a) SC – F55 $r = 0.338$, $n = 59$, $P = 0.009$; (b) SC – F89 $r = -0.039$, $n = 59$, $P = 0.771$; (c) F89 – F55 $r = -0.091$, $n = 58$, $P = 0.496$.

be more attractive in F55 light than in nature and the other treatments. F89 also shows strong differential cone stimulation (Fig. 1), but it mostly stimulates the L cones. This means that guppies will be least sensitive to long wavelength light and the orange spots will be relatively difficult to see, reducing their conspicuousness. SC provides an environment (early/late, Endler 1993a,b) similar to the time of maximum courtship (Fig. 1, Endler 1987, 1991), but this naturalistic environment may not produce as high variation in contrast as the more artificial stimuli F89 and F55.

Males did not change their rate of courtship display in response to light spectra typical of different times of day or microenvironments. Since the irradiance was roughly equal under the three treatments this is consistent with courtship display rate being related to irradiance intensity (Endler 1987) rather than spectral composition. Endler (1987), Reynolds *et al.* (1993) and Long & Rosenqvist (1998) have shown that male courtship is less frequent under high light intensities typical of midday and more frequent under the lower intensities typical of early or late conditions. Female responsiveness to male courtship was also highest under low light intensity, and larger males (size was the best predictor of attractiveness in Reynolds' experiments) reduced their courtship rate under increased light (Reynolds 1993). This finding may result from a reduced benefit of being attractive as female responsiveness decreases under higher light intensities and/or a greater cost to larger males being conspicuous to predators (Reynolds 1993). In our study, larger males courted less often in all treatments, even though our light intensities are at the lower end of the natural daytime light intensity ranges. This is consistent with no differential costs or benefits of high courtship rates to males of different sizes under the three light treatments, because the equal intensities may be perceived as equal (and low) predation risk environments.

Neither Endler (1987) nor Reynolds *et al.* (1993) found differences in the rate of attempted sneak copulations at different light intensities. Our finding that males attempt more sneaks under the SC and F89 light suggests, however, that males in light environments in which females are better able to judge attractiveness (F55) sneak less and those in a less attractive environment (F89 and SC) sneak more. Taken together with published evidence on the effects of light intensity (Endler 1987; Reynolds 1993; Reynolds *et al.* 1993; Long & Rosenqvist 1998), our results imply that diel variation in male and female mating strategies in guppies is due to a combination of light intensity and spectral composition, and that these factors affect different components of courtship and sexual selection.

Male mating behaviour and female choice in guppies have been demonstrated to covary with a number of environmental and social factors, including the colour of ambient light (Long & Houde 1989; Brooks & Caithness 1995b), operational sex ratio (Jirotkul 1999a), population density (Rodd & Sokolowski 1995; Jirotkul 1999b), the population of origin of tank mates (Rodd & Sokolowski 1995), and the mean and distribution of attractiveness or ornamentation among males in the tank (Brooks & Caithness 1995a; Rosenqvist & Houde 1997; Jirotkul 2000). The differences in attempted sneak copulations among light spectrum treatments that we

Table 3 Analysis of covariance between male attractiveness under the three light treatments and measures of male ornamentation. Attractiveness scores are standardized for each group of females

	Slope comparisons						Common slope		
	Treatment × trait			Rep(treatment) × trait			Trait		
	d.f.	*F*	*P*	d.f.	*F*	*P*	d.f.	*F*	*P*
Body area	2, 15	3.84	0.045	15, 144	0.83	0.639	1, 144	–	–
Tail area	2, 15	0.17	0.849	15, 144	1.31	0.206	1, 144	0.56	0.464
Black*	2, 15	0.95	0.410	15, 144	0.91	0.512	1, 144	1.45	0.247
Fuzzy black*	2, 15	1.05	0.375	15, 144	1.71	0.055	1, 144	3.20	0.094
Orange*	2, 15	0.70	0.512	15, 144	0.85	0.620	1, 144	4.27	0.057
Iridescence*	2, 15	1.19	0.306	15, 144	1.658	0.066	1, 144	1.15	0.301
Total spot number	2, 15	1.97	0.174	15, 144	1.05	0.410	1, 144	0.14	0.709

*Proportion of total body area.
–, indicates common slopes may not be assessed because of significant slope heterogeneity.

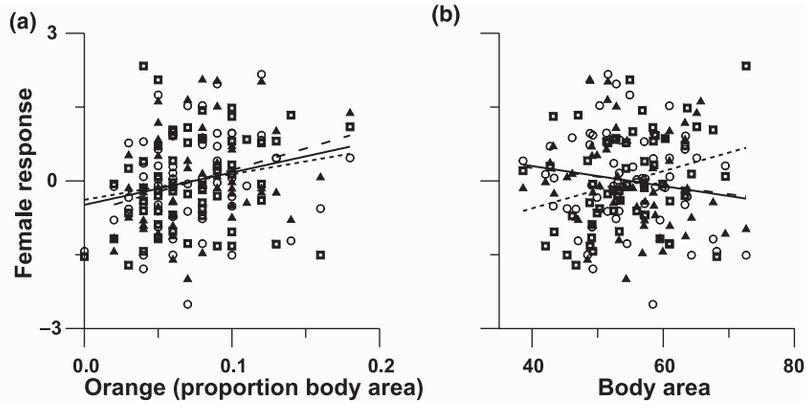

Figure 4 Relationship between male attractiveness (standardized for each group of females) and (a) proportion of body area covered by orange spots and (b) body area, under the three light spectrum treatments. SC light treatment: open circle, solid line; F89 light treatment: open square, short dashed line; F55 light treatment: closed triangle, long dashed line. The lines for SC light and F55 light treatments overlap almost entirely.

Table 4 Multiple regression models of attractiveness under the three light spectrum treatments

	Standardized coefficient (β)		
	SC	F89	F55
Body area		0.239	
Tail area		0.260	0.176
Black†			0.253
Fuzzy black†	−0.177		
Iridescence†			
Orange†	0.258*	0.373*	0.377*
Yellow†			
Total spot number			−0.222
Model			
r^2_{adj}	0.074	0.212	0.124
F	3.37	6.29	3.10
d.f.	2, 57	3, 56	4, 55
P	0.041	0.001	0.023

*$P < 0.05$.
†Proportion of body area.

demonstrate here are consistent with the extreme documented plasticity of male mating behaviours in this species.

Male fitness

The repeatability (0.104 ± 0.08) of male attractiveness across treatments was low relative to estimates from studies in which repeat measures were made under consistent lighting conditions. These range from 0.32 ± 0.05 (Brooks 1996) to 0.47 ± 0.11 (Kodric-Brown & Nicoletto 1997). The low repeatability of male attractiveness may be due to the fact that females preferred different males under F89 light from those that they preferred under SC and F55 light. We are not aware of any other studies in any species that explicitly demonstrate that male attractiveness is uncorrelated across signalling environments, although this (or a more extreme situation in which attractiveness in different signalling environments trade-off) could be an important process maintaining variation in male quality and signals. If males' attractiveness changes with light environment, then

male fitness estimates from single light environments (e.g. in the lab) may give misleading estimates of fitness, and underestimate the ability of a system to maintain variability in male traits. In addition, it also implies that environmental changes should result in changes in which groups of male traits should be more attractive and spread.

The significant, highly consistent (across treatments) female preference for males with a large proportion of the body covered by orange pigment indicates that orange coloration is likely to be under directional selection under a range of signalling environments in the field. Two earlier studies have demonstrated significant differences in preference for orange coloration among manipulated incident light spectra (Long & Houde 1989; Brooks & Caithness 1995b). In these studies, treatment effects were strongest under orange light when preferences disappear because orange spots are indistinguishable from the background body colour. Under blue and green light, female preferences for orange area were indistinguishable from control (full spectrum) preferences (Long & Houde 1989; Brooks & Caithness 1995b). Our treatments used naturalistic spectra with low chromaticity, and therefore did not mask orange colour as much as the high-chroma spectra used by Long & Houde (1989) and Brooks & Caithness (1995a,b). Females appear able to identify and respond positively to males bearing large areas of orange under these conditions. There are two possible explanations. First, our low-chroma irradiance spectra will affect the radiance spectra coming off the male colour patterns far less than the high-chroma irradiances used previously (Long & Houde 1989, Brooks & Caithness 1995a,b), and are therefore much less likely to change females' perception of the male colour patterns (Endler 1991, 1992, 1993b). Secondly, guppies may have some sort of colour constancy, a neural mechanism that keeps colour perception relatively independent of irradiance. Colour constancy only breaks down in highly chromatic light (e.g. Long & Houde 1989; Brooks & Caithness 1995a,b), but is likely to remain in the low chromaticity lights that we used. The simplest models (e.g. Vorobyev & Osorio 1998) postulate colour constancy arising out of light adaptation of the cones; in F89 light the L cones would be less sensitive than the other cones, making the colour signal (L – M cone difference) for orange depend more on the reflectance of the orange spot than the irradiance. However, in humans, it is clear that colour constancy depends also upon higher order processing (Kraft & Brainard 1999), and the complexity of the visual background pattern (Kraft *et al.* 2002). If guppies have similar neural processing, then this would also explain the light-independence of the preference for orange.

It is likely that both colour constancy and the low-chroma light spectra make some preferences unlikely to differ among light environments. Male traits other than orange may be under different sexual selection in different signalling environments. This may be the case for body size, in particular, where females preferred larger males under F89 light and smaller males under F55 and SC spectrum light. We do not know whether or not the lack of correlation in male attractiveness between the F89 treatment and the other two treatments is solely due to the opposing preferences for large/small males. The other subtle differences among treatments in multivariate female choice may be of biological significance too, and might result in sufficiently different selection on different ornaments in different environments to maintain some of the prodigious variability in these ornaments. Such subtle differences in direct selection may have major implications for the actual selection response due to the complexities added by indirect selection due to genetic correlations among traits (Brooks & Endler 2001).

Maintenance of polymorphism

The effects of variation in light spectrum documented here, and other effects of differing light intensity and water transmission properties (Endler 1987, 1991), may contribute to within-population variation in male ornamentation in four ways. First, the importance of sexual selection relative to predator-induced selection for crypsis may vary across the day and among microenvironments within streams (Endler 1980, 1983, 1987, 1991). Second, the relative importance of different sexually selective processes, including female choice, male sneak copulation and associated sperm competition, are also likely to covary with spatial and temporal differences in the light environment associated with variation in predation risk (e.g. Endler 1987). Third, the visual background (gravel size and colour) also covaries with light environment and predation risk (Endler 1980), and a large number of different colour patterns can be equally cryptic, or equally conspicuous on these complex visual backgrounds (Endler 1980, 1988). If predation and various sexual selection processes impose opposite directions of net selection on male traits, then variation within streams, and even diurnal variation in the relative importance of these processes, may allow the persistence of substantial variability – there may even be several 'most successful' phenotypes that trade-off these multiple, conflicting, adaptive needs (Endler 1980, 1988; Blows *et al.*, in press).

The fourth possibility is that different phenotypes may be favoured by the same sexually selective process under different conditions. We have considered the case of female mate choice here, and the evidence is somewhat divided. It appears that sexual selection by female mate choice is likely to favour greater amounts of orange under all three light spectra. It is thus unlikely that fluctuations in direct selection maintain variation in orange coloration. Body size, however,

may be under positive selection from choosy females under F89 light but negative selection under F55 and SC spectrum light. This effect and subtle differences in the pattern of multivariate female preference under different lighting conditions appear to be present and may explain why male attractiveness was uncorrelated between SC and F89 and between F89 and F55 light treatments.

Studies that estimate mating success and thus directional and quadratic selection gradients in different environments will help us assess these three sets of possibilities and to explore whether there are trade-offs between overall male fitness among signalling environments and whether there are opposing forces of selection operating in these environments.

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REFERENCES

Andersson, M. (1994). *Sexual Selection*. Princeton University Press, Princeton, NJ.

Barton, N.H. & Turelli, M. (1989). Evolutionary quantitative genetics: how little do we know? *Annu. Rev. Genet.*, 23, 337–370.

Becker, W.A. (1992). *Manual of Quantitative Genetics,* 5th edn Academic Enterprises, Pullman, WA.

Boughman, J.W. (2001). Divergent sexual selection enhances reproductive isolation in sticklebacks. *Nature*, 411, 944–947.

Blows, M.W., Brooks, R. & Kraft, P.G. (in press). Exploring complex fitness surfaces: multiple ornamentation and polymorphism in guppies. *Evolution*.

Brooks, R. (1996). Copying and the repeatability of mate choice. *Behav. Ecol. Sociobiol.*, 39, 323–329.

Brooks, R. & Caithness, N. (1995a). Does a male's attractiveness to a female depend on her previous experience? S. *Afr. J. Sci.*, 91, 156–158.

Brooks, R. & Caithness, N. (1995b). Female guppies use orange as a mate choice cue: a manipulative test. *S. Afr. J. Zool.*, 30, 200–201.

Brooks, R. & Endler, J.A. (2001). Direct and indirect sexual selection and quantitative genetics of male traits in guppies (*Poecilia reticulata*). *Evolution*, 55, 1002–1015.

Draper, N. & Smith, H. (1981). *Applied Regression Analysis,* 2nd edn. John Wiley, New York.

Endler, J.A. (1980). Natural selection on color patterns in *Poecilia reticulata*. *Evolution*, 34, 76–91.

Endler, J.A. (1983). Natural and sexual selection on color patterns in poeciliid fishes. *Environ. Biol. Fishes*, 9, 173–190.

Endler, J.A. (1987). Predation, light intensity and courtship behaviour in *Poecilia reticulata* (Pisces: Poeciliidae). *Anim. Behav.*, 35, 1376–1385.

Endler, J.A. (1988). Frequency-dependent predation, crypsis, and aposematic coloration. *Philos. Trans. R. Soc. Lond. Ser. B*, 319, 505–523.

Endler, J.A. (1991). Variation in the appearance of guppy color patterns to guppies and their predators under different visual conditions. *Vision Res.*, 31, 587–608.

Endler, J.A. (1992). Signals, signal conditions, and the direction of evolution. *Am. Nat.*, 139, S125–S153.

Endler, J.A. (1993a). The color of light in forests and its implications. *Ecol. Monogr.*, 63, 1–27.

Endler, J.A. (1993b). Some general comments on the evolution and design of animal communication systems. *Philos. Trans. R. Soc. Lond. Ser. B*, 340, 215–225.

Endler, J.A. (2000). Evolutionary implications of interactions between animal signals and the environment. In: *Adaptive Significance of Signalling and Signal Design in Animal Communication* (eds Espmark, Y., Amundsen, T. & Rosenqvist, G.). Tapir Publishers, Trondheim, Norway, pp. 11–46.

Endler, J.A. & Houde, A.E. (1995). Geographic variation in female preferences for male traits in *Poecilia reticulata*. *Evolution*, 49, 456–468.

Fleishman, L.J. & Endler, J.A. (2000). Some comments on visual perception and the use of video playback in animal behavior studies. *Acta Ethol.*, 3, 15–27.

Houde, A.E. (1987). Mate choice based upon naturally occurring colour-pattern variation in a guppy population. *Evolution*, 41, 1–10.

Houde, A.E. (1997). *Sex, Color and Mate Choice in Guppies*. Princeton University Press, Princeton, NJ.

Jirotkul, M. (1999a). Operational sex ratio influences female preference and male–male competition in guppies. *Anim. Behav.*, 58, 287–294.

Jirotkul, M. (1999b). Population density influences male–male competition in guppies. *Anim. Behav.*, 58, 1169–1175.

Jirotkul, M. (2000). Male trait distribution determines alternative mating tactics in guppies. *J. Fish Biol.*, 56, 1427–1434.

Kirkpatrick, M. & Ryan, M.J. (1991). The evolution of mating preferences and the paradox of the lek. *Nature*, 350, 33–38.

Kodric-Brown, A. & Nicoletto, P.F. (1997). Repeatability of female choice in the guppy: response to live and videotaped males. *Anim. Behav.*, 54, 369–376.

Kraft, J.M. & Brainard, D.H. (1999). Mechanisms of color constancy under nearly natural viewing. *Proc. Nat. Acad. Sci. USA*, 96, 307–312.

Kraft, J.M., Maloney, S.I. & Brainard, D.H. (2002). Surface-illuminant ambiguity and color constancy: effects of scene complexity and depth cues. *Perception*, 31, 247–263.

Long, K.D. & Houde, A.E. (1989). Orange spots as a visual cue for female mate choice in the guppy (*Poecilia reticulata*). *Ethology*, 82, 316–324.

Long, K.D. & Rosenqvist, G. (1998). Changes in male guppy courting distance in response to a fluctuating light environment. *Behav. Ecol. Sociobiol.*, 44, 77–83.

Pomiankowski, A. & Møller, A.P. (1995). A resolution of the lek paradox. *Proc. R. Soc. Lond. Ser. B*, 260, 21–29.

Quinn, G.P. & Keough, M.J. (2002). *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge.

Reynolds, J.D. (1993). Should attractive individuals court more? Theory and a test. *Am. Nat.*, 141, 914–927.

Reynolds, J.D., Gross, M.R. & Coombs, M.J. (1993). Environmental conditions and male morphology determine alternative mating behaviour in Trinidadian guppies. *Anim. Behav.*, 45, 145–152.

Rodd, F.H. & Sokolowski, M.B. (1995). Complex origins of variation in the sexual behaviour of male Trinidadian guppies, *Poecilia reticulata*: interactions between social environment, heredity, body size and age. *Anim. Behav.*, 49, 1139–1159.

Rosenqvist, G. & Houde, A. (1997). Prior exposure to male phenotypes influences mate choice in the guppy, *Poecilia reticulata*. *Behav. Ecol.*, 8, 194–198.

Rowe, L. & Houle, D. (1996). The lek paradox and the capture of genetic variance by condition dependent traits. *Proc. R. Soc. Lond. Ser. B*, 263, 1415–1421.

Ryan, M.J. & Wilczynski, W. (1991). Evolution of intraspecific variation in the advertisement call of a cricket frog (*Acris crepitans*, Hylidae). *Biol. J. Linnean Soc.*, 44, 249–271.

Taylor, P.D. & Williams, G.C. (1982). The lek paradox is not resolved. *Theor. Popul. Biol.*, 22, 392–409.

Vorobyev, M. & Osorio, D. (1998). Receptor noise as a determinant of colour thresholds. *Proc. R. Soc. Lond. B*, 265, 351–358.

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Chapter 9

The complex social environment of female house mice (*Mus musculus domesticus*)

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The complex social environment of female house mice (*Mus domesticus*)

BARBARA KÖNIG AND ANNA K. LINDHOLM

Introduction

Conspecifics are a major environmental factor for most organisms. In sexually reproducing species they are not only mating partners, but also partners in competitive and cooperative interactions. In a social species, females therefore interact with males as potential mating partners and with other females as potential social and cooperation partners. On the other hand, conspecifics are also competitors for limited resources when living in close proximity. Conflicts are thus inevitable when females form groups, despite any benefits of group living (Alexander, 1974; Emlen and Oring, 1977; Sterck *et al.*, 1997). In recent reviews, Clutton-Brock (2009) as well as Stockley and Bro-Jørgensen (2010) emphasize the lack of knowledge about female competitive strategies, despite increasing evidence for intrasexual competition and sexual conflict among females. Female mammals regularly compete among themselves over access to resources such as food, breeding territories, nest sites, and shelter. In species with multi-female groups, they may further compete over breeding (dominance) rank, assistance with offspring care, the number of offspring raised, protection of offspring during babysitting, or social thermoregulation. Females may also compete over access to mating partners in the course of a single breeding cycle, and competitive behaviour among females generally includes aggression, weaponry, alliance formation, cooperation, and the inhibition of other females' reproduction (Rosenqvist and Berglund, 1992; Clutton-Brock, 2009; Stockley and Bro-Jørgensen, 2010). However, few data are available regarding whether differential access to resources (including mating partners) leads to differences in reproductive success, and whether social interactions affect the outcome of such reproductive competition.

The above strategies, which females may use during intrasexual competition, are less conspicuous than those used by males, and this difference has been linked to higher parental investment and lower potential reproductive rates in female

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mammals (see Clutton-Brock, 2009; Stockley and Bro-Jørgensen, 2010). Nevertheless, any selective influence of reproductive competition will tend to result in the development of traits that allow for more competitive interactions. Such a process has been termed 'social selection' by West-Eberhard (1983, 1987), with sexual selection considered a subset. Social selection refers to the fact that an individual's fitness is not only determined by its own phenotype but also by the phenotype of a social partner. The potential for social selection exists whenever individual fitness varies as a result of social interactions, both in a cooperative and competitive context. In mammals in which females live in social groups, social interactions thus may provide the opportunity for the evolution of traits that improve reproductive competitiveness as well as social partner choice in females, with the prediction that interactants experience variance in fitness. So far, fitness consequences of female social interactions have only been described for primates (Silk *et al.*, 2003) and for house mice (Weidt *et al.*, 2008).

House mice are a tractable species for studies of female social interactions and female intrasexual competition, as well as the potential for reproductive competition to affect a female's social environment. Here, we first review factors that influence female–female social interactions during reproductive cooperation and competition. We then present new data from a free-living population in Switzerland showing seasonal effects on female reproductive competition and social bonding, and analyse whether differences in degree of reproductive competition and in current reproductive state affect social interactions of adult females.

Reproductive competition and cooperation in female house mice

Female house mice (*Mus domesticus*) live in social groups, and field as well as laboratory studies have illustrated their high potential for cooperation and competition over reproduction. House mice have a flexible social structure, but typically they live in small groups consisting of a dominant male, one or several adult females with their litters and several subordinate mice of both sexes (DeLong, 1967; Lidicker, 1976; Bronson, 1979; Berry, 1981; Singleton, 1983; Gray *et al.*, 2000). House mice are plural breeders, with several breeding females per group. Within groups, adult females contribute to territorial defence (Crowcroft, 1966; Latham and Mason, 2004) and cooperate in some kinds of communal care, such as babysitting, social thermoregulation, or defence of pups. The most conspicuous example of cooperation, however, is non-offspring nursing (for a review, see König, 2006). Such non-offspring nursing occurs when two – or, rarely, more – females pool their litters in a communal nest and

indiscriminately nurse both own- and non-offspring (Southwick, 1955; Sayler and Salmon, 1971; König, 1989, 1993; Manning *et al.*, 1995).

Kept under standardized conditions in the laboratory or in semi-natural enclosures, non-offspring nursing is an integral part of the reproductive behaviour of female house mice in egalitarian groups. Communal or non-offspring nursing (also referred to as allosuckling or allonursing) has been described for approximately 70 mammalian species across 12 orders, and for reproducing and non-reproducing females; nevertheless, in only 10% of such species were non-offspring nursed as much as own young, as is the case in house mice (reviewed in Packer *et al.*, 1992; Jennions and Macdonald, 1994; König, 1997, 2006; Lewis and Pusey, 1997; Solomon and French, 1997; Hayes, 2000).

Communal nursing in house mice has been studied intensively in the laboratory with direct descendants of wild-caught animals. When two females establish an egalitarian reproductive relationship, communal nursing increases both partners' individual lifetime reproductive success in comparison to females rearing litters solitarily (König, 1993, 1994a). However, the probability for such cooperation is highest when a female shares a nest with a familiar sister to form a low-skew society (König, 1994a, b, 1997, 2006). As a consequence, non-offspring nursing of female house mice in pairs with egalitarian reproduction is adaptive, and involves mutualistic direct fitness benefits. With increasing group size (three adult females per group), on the other hand, reproductive skew increases towards despotic relationships and individual reproductive success decreases below that of a solitary female. The reason why females differ in individual reproductive success as a function of group size and relatedness is that females differ in their probability to reproduce and successfully wean young within their lifetime, due to competition over reproduction, despite communal nesting and communal nursing (König, 1994a, b, 2006).

Individual fitness thus varies as a result of social interactions, and direct benefits of cooperation seem to stabilize non-offspring nursing among female house mice. Spatial proximity and nest sharing usually precede breeding associations in house mice (Manning *et al.*, 1995; Dobson *et al.*, 2000; Hayes, 2000; Rusu and Krackow, 2004), while spatial intolerance and unstable dominance relationships prior to the start of reproduction strongly impair cooperation in communal nests (Rusu and Krackow, 2004). Females may therefore be expected to carefully choose partners for subsequent breeding to avoid fitness loss, and establish social bonds to such preferred partners. In accordance with this hypothesis, female house mice display non-random preferences for social partners when kept in groups of unrelated females in semi-natural enclosures (Weidt *et al.*, 2008). Females that were afterwards allowed to live with previously preferred social partners had a higher probability to reproduce and a significantly higher lifetime reproductive success compared to females living with previously non-preferred partners. This suggests

that females generally associate with social partners with whom they have a low potential for reproductive competition (Weidt *et al.*, 2008).

In natural populations and in enclosures, female house mice spatially associate and communally nest with kin (Wilkinson and Baker, 1988; Dobson *et al.*, 2000; Dobson and Baudoin, 2002; Rusu and Krackow, 2004; Rusu *et al.*, 2004). Familiarity during juvenile development (as occurs among siblings) is of importance for such female–female social interactions (König, 1994b; D'Amato, 1997). This is in contrast to female mate choice, where dominance status (Hayashi, 1990; Drickamer, 1992) or genotypic cues (MHC complement – Yamazaki *et al.*, 1976; or *t* haplotype – Lenington and Egid, 1989) are of main relevance. This suggests that for social partner choice direct information about a potential partner's behaviour or physiological status is more important than genetic relatedness (see also König, 1994b, 2006). Nevertheless, choosing a familiar female for social cooperation may result in close association with kin. Incidences of female competition over reproduction, mainly expressed through overt aggression, reproductive inhibition of other females or infanticide of non-offspring, on the other hand, have been typically described for unfamiliar and unrelated females (Hurst, 1987; König, 1994a; Palanza *et al.*, 1996, 2005; Rusu and Krackow, 2004).

The potential for female house mice to establish individualized social bonds among group members is therefore high. House mice mainly use odours (for a review see Stopka *et al.*, Chapter 8 in this volume), but also ultrasonic vocalizations (for review see Musolf and Penn, Chapter 10 in this volume) for intraspecific communication and individual identification, and familiar individuals can recognize each other after a separation period of at least seven days (Hurst, 1990; D'Amato, 1997; D'Amato and Moles, 2001; Nevison *et al.*, 2003).

Seasonal reproduction in house mice

Reproduction in house mice is assumed not to be obligatorily seasonal. Commensal populations usually breed all year, and feral populations breed non-seasonally in some locations, but are seasonal in others (reviewed in Latham and Mason, 2004). In his extensive review of the reproductive ecology of house mice, Bronson (1979) concluded that the interaction between caloric deprivation and the metabolic responses to cold exposure seem to be the most likely candidate for producing a seasonal interruption of the potential of mice for continuous breeding. During cold periods, reproduction is often reduced, although cold-adapted house mice have bred in frozen carcasses in deep freezer houses in the harbour of London (Laurie, 1946). Seasonal variability in commensal barn populations therefore may allow a comparison of periods with high

reproductive activity and a potentially high degree of reproductive competition, with periods of relatively low reproductive activity and thus low competition among females.

From free-living populations, however, little information is available on the social life of female house mice and how it is affected by differences in female competition over reproduction and by female bonding. Here, we illustrate the complexity of the social environment of a wild female house mouse, using new data from our long-term study of a free-living population in a barn in Switzerland. We were specifically interested in the following questions:

- Is there evidence that female reproductive competition varies seasonally?
- Does seasonality in reproductive competition affect the social environment of females?
- Does current reproductive state influence female–female social interactions independently of seasonal effects?

Assuming that (1) females display social partner preferences; (2) individual reproductive success is highest in pairs of females and decreases with increasing group size; and (3) high reproductive activity implies high reproductive competition, reproducing females are expected to choose smaller group sizes during periods of high reproductive activity.

Study population of wild house mice

Our study population was initiated in autumn 2002 by Andrea Weidt and Barbara König in a former barn, situated at the border of a forest near Illnau, Kanton Zürich, Switzerland. For a house mouse, a barn is a natural habitat. House mice in Europe occur in anthropogenic habitats, such as grain stores and farm buildings, with feral populations generally restricted to islands (Pocock *et al.*, 2004). This has been the case for a long time. Archaeological and palaeontological evidence shows that house mice were commensal with humans by 8000 BC, coinciding with new farming practices such as large-scale grain storage (Cucchi *et al.*, 2002), and that house mice colonized western Europe, commensal with humans, about 2000 years ago (Cucchi *et al.*, 2005).

Our study population was seeded with 12 wild-caught individuals, six from each of two nearby demes (altogether four males and eight females, caught at two farmhouses situated within 5 km of the barn) in November 2002, and has since August 2003 been open to immigration and emigration (after successful reproduction of the founder individuals). Long-distance dispersal out of the barn occurs, as marked mice have been recovered approximately 1 km away.

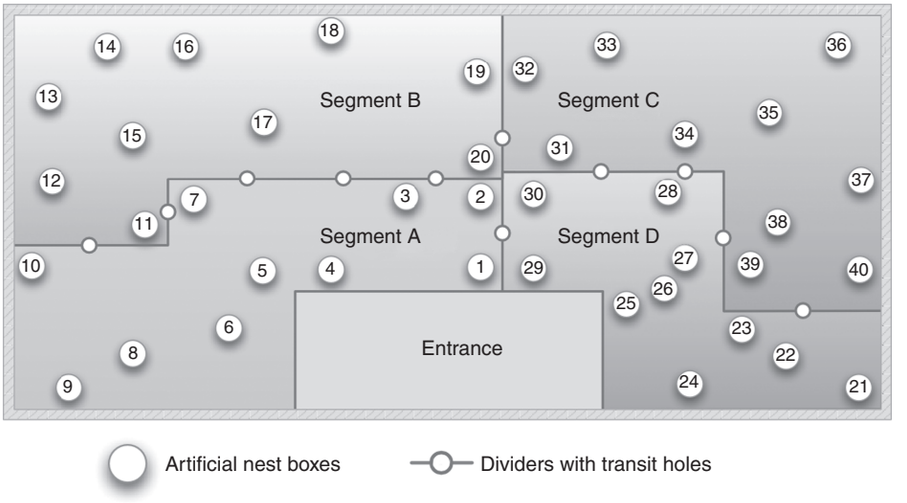

Figure 5.1 Schematic drawing of the floor of the barn (floor space 72 m²) with 40 nest boxes. Dividing walls are aluminium, 75 cm high, with 11 passages, separating the area into four quarters; next to the entrance door into the barn is a separate area for storage of equipment and for handling the mice – this is also accessible to the animals. Not shown are 2–4 feeding and drinking sites each per quarter, and further structuring of the floor with bricks and smaller wooden and plastic barriers or hides.

The barn is divided into four quarters by aluminium plates 75 cm in height, with 11 small holes allowing the passage of mice (Fig. 5.1). The floor of the barn consists of concrete, and is covered by commercial rodent bedding, and littered with bricks and wooden and plastic barriers to provide internal structure and shelters (not shown in Fig. 5.1). The mice can access all parts of the barn, and can leave it under the roof or through holes in the walls (the walls consist partly of bricks and wood). They nest in 40 artificial nest boxes (ten per quarter) and are provided with straw as nesting material. The interiors of the nest boxes are accessible to us, so offspring can be counted and measured. Food, a 50–50 mixture of oats and commercial rodent food made by Haefliger AG, is provided *ad libitum* at 9–10 feeding trays (at least two per quarter), as is water (3–4 drinking sites per quarter). Availability of food at our study site is less than would be available in grain stores and many barns, but is not limiting. We therefore consider the availability of food within the natural range. The barn itself is free of predators, but not of parasites; mice are exposed to predators, including foxes, badgers, house cats, and birds of prey, whenever they exit the barn.

Male and female mice of minimally 18 g living in the barn are implanted with a transponder (RFID tag; trovan® ID 100, 0.1 g weight, 11.5 mm length, 2.1 mm

diameter) by a trained and licensed animal care technician. Implanting transponders into smaller mice results in a high percentage of tags lost. Our method nonetheless allows tagging of all reproducing females, as no pregnant or lactating females weighing less than 19 g have been detected (2006–2010). The transponders provide a unique identity number for each mouse and a means to monitor mice remotely by transponder readers.

In 2003, 2004, and since 2006 until now, all nest boxes and shelters have been monitored weekly for the presence of tagged mice (which is done with a handheld transponder reader placed outside the nest box or a shelter) and for the presence of new litters. During 2005, monitoring was less frequent. The age of pups is estimated, and all litters are measured when they are 13 days of age (range 12–14 days; day of birth of a litter is considered as day 1). We sex the pups and take individual body weight. We refer to the number of pups at day 13 as weaning litter size, because pups are not yet mobile and thus do not mix with other litters by themselves. At 14 days of age, pups open their eyes and are mobile. They begin to eat solid food at 17 days and are weaned when 21–23 days old (dependent on litter size; smaller litters are weaned earlier; König and Markl, 1987). Offspring mortality between days 13 and 17 is almost absent in the laboratory, and we consider litter size at day 13 as a good approximation of weaning litter size also in our barn population.

In addition, at approximately seven-week intervals, comprehensive trapping has been conducted to monitor the adult population (population monitoring). Every mouse is weighed and females are examined for reproductive state (characterized as pregnant and/or lactating according to the swelling of the body and the appearance of the teats), and those adults lacking transponders are tagged. We also monitor the population for remains of deceased mice. Here, we analyse 29 population-monitoring events over a period of four years, from April 2006 until March 2010.

In May 2007 we installed permanent transponder readers in the tunnels that provide entrances to the nest boxes (two antennas per tunnel, which allows us to discriminate between a mouse entering or leaving a nest box). These readers connect to a computer and continuously track movements of tagged mice into and out of nest boxes. This provides 24-hour information on movements and social affiliations of adult mice, and makes breeding females and males easy to locate. Here we include data from 1 January 2008 to 31 December 2009 on tagged females.

Seasonal variability in reproduction

The number of adult males and females in our study population increased from an average (± standard error) of 29.6 ± 4.4 males (range 11–57) and 38.9 ± 3.0 females (range 27–55), respectively, in the 12 months from April 2006, to 69.7 ± 8.3 males (range 55–88) and 70.7 ± 4.8 females (40–100) in the 12

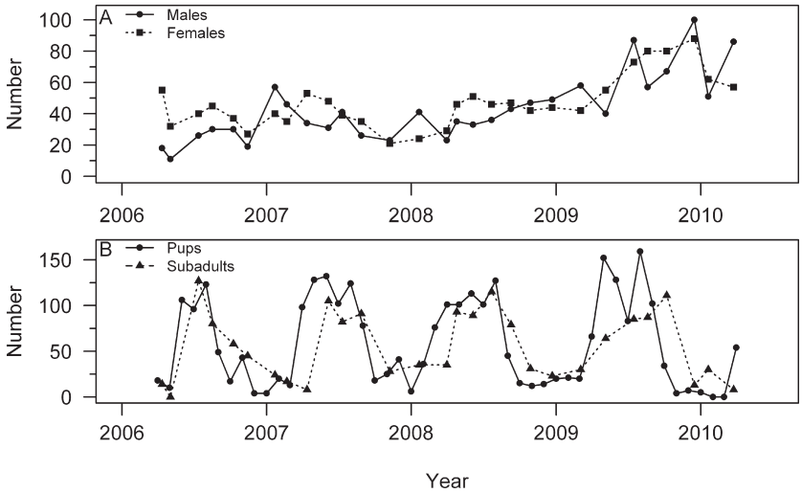

Figure 5.2 (a) Adult males and adult females (body weight at least 18 g) and (b) subadults (weaned offspring of less than 18 g) registered during population-monitoring events over a period of four years (April 2006 to March 2010; $N = 29$); (b) further shows the total number of pups sampled per month at the age of 13 days ($N = 48$).

months from April 2009 (Fig. 5.2a). No seasonal effects on adult sex ratio were obvious; however, during the first three years (2006–2008), the number of adult females was higher than the number of adult males during the summer, with the reverse effect during the winter. The number of pups weaned (13 days of age) and of subadults (weaned individuals up to a body weight of 17.5 g), on the other hand, showed marked seasonal effects, with peaks in reproductive output at 12-month intervals, during late spring/early summer (beginning April/May; Fig. 5.2b). The number of subadults registered during a single population-monitoring event reached values of up to 127 during mid-summer. Although reproduction rarely entirely stopped during the winter, it was typically drastically reduced, beginning in September/October.

Seasonality in reproduction was also reflected in the proportion of reproductive females present in the population (Fig. 5.3a). This measure may reflect female reproductive activity better than pup production, as only pups surviving to the age of 13 days are included in the latter measure. However, it may fail to include non-lactating females in early pregnancy. Generally, the proportion of females pregnant and/or lactating was lower during winter (October to March) than during summer (April to September; binomial GLM, $z_{1,27} = 9.84$, $p < 0.0001$; statistics were performed using R version 2.12.2; R Core Development Team, 2011). This is

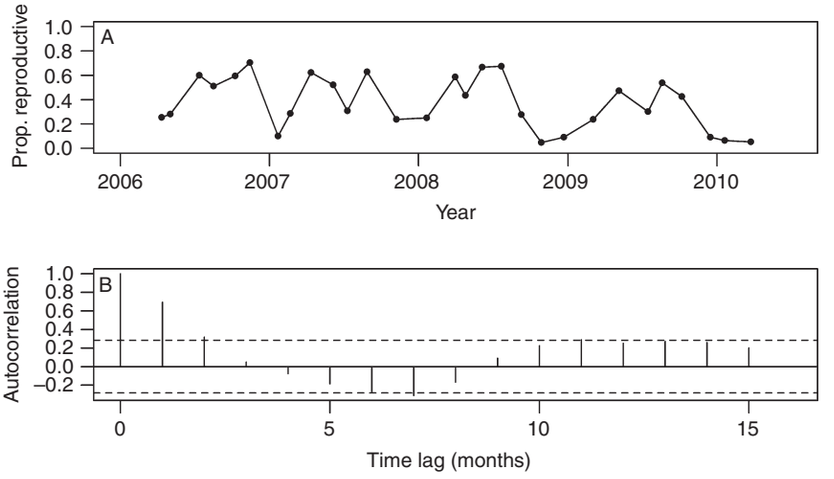

Figure 5.3 Proportion of adult females classified as reproductive (pregnant and/or lactating) during population-monitoring events; (a) over a period of four years (April 2006 to March 2010; $N = 29$); (b) correlogram of temporal autocorrelation. The dashed line gives the 95% confidence interval. Vertical bars crossing the confidence interval indicate significant autocorrelations.

illustrated by the significant negative autocorrelation of the proportion of reproductively active females after a seven-month lag time (Fig. 5.3b), using linear interpolation to estimate missing monthly values (as monitoring sessions were less frequent than once per month). Peaks of positive correlations after 11–14 months indicate an approximate annual cycle in reproduction.

During the study period, the proportion of females showing reproductive activity decreased with time (binomial GLM, $z_{1,27} = 2.10$, $p < 0.05$). As numbers of adult females showed an increase with time (linear regression $t_{1,27} = 4.23$, $p < 0.001$), but numbers of reproductively active females did not (linear regression $t_{1,27} = 0.12$, $p < 0.91$), the decrease in proportion of reproductive females over time could be due to increased female competitive interactions. We used a generalized linear model to test for an effect of the number of adult females present on the proportion of females showing reproductive activity (Fig. 5.4). To remove the effect of seasonality in breeding, we averaged across 12-month intervals, and we log-transformed the average number of females present to avoid over-dispersion of errors. Indeed, reproductive activity significantly declined with increasing numbers of females present (binomial GLM, $z_{1,2} = 5.25$, $p < 0.001$).

To summarize, reproductive skew among adult females was substantial even during periods of favourable breeding conditions during summer. The number

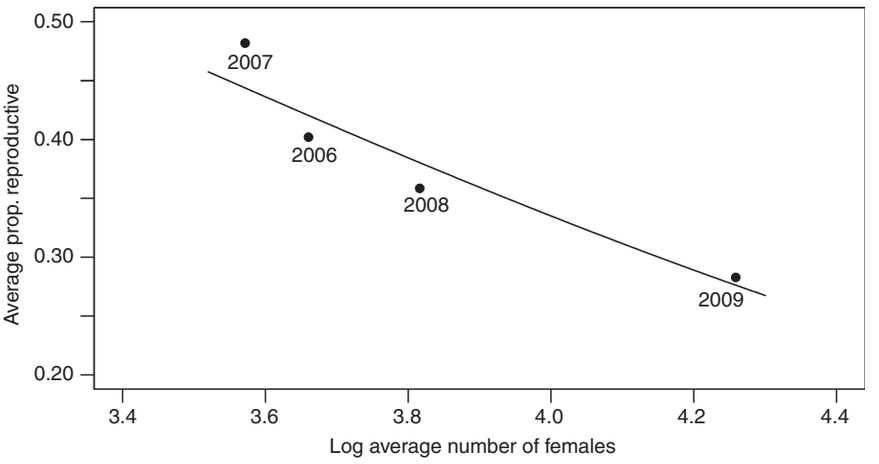

Figure 5.4 Average proportion of females classified as reproductive (pregnant and/or lactating), as a function of the log-transformed average number of females present (points), per year, with generalized linear model prediction (line).

of reproductively active females did not change significantly over the study period, nor did the annual total of pups weaned. However, the number of non-breeding adult females in the population increased over time, suggesting increasing female competition over reproduction, especially during the summer. In winter, however, under conditions of unfavourable, cold temperatures, a female's individual condition or physiology, and not necessarily reproductive competitiveness against other females, might determine whether she is able to reproduce. Reduced food and nesting material availability can be excluded in our study population as causal factors for low breeding during winter, as suggested for other wild populations (Laurie, 1946; Berry, 1968; Randall, 1999). Instead, cold and its ensuing influence on metabolism may be the greatest hazard to a female (see also Lynch, 1992).

Male condition also supports our conclusion that the strength of intrasexual selection varies seasonally. Given that wounds in males are typically inflicted during intrasexual competition over access to females, only 8.8% of adult males examined during population-monitoring events had fresh wounds on their body, head, legs, or tail in winter, but 21% were wounded during summer.

Whatever the cause of the observed seasonality in reproduction, we expect lower female competition during winter than during summer, leading to the next question of seasonal differences in female–female interactions and female bonding.

Seasonal variability in nest box use and in social interactions among females

Female nest box use and social behaviour were analysed from a two-year dataset of transponder readings in the nest boxes. For each tagged female we calculated the following monthly data (beginning with the month of tagging for females implanted after January 2008, and excluding the month during which a female died or disappeared from the barn): cumulative number of nest boxes visited; cumulative number of female partners; and cumulative number and mean duration of interactions. Social interactions and female partners were defined according to meetings in the same nest box. As soon as any two females simultaneously visited the same nest box, they were considered as partners and the meeting as a social interaction. Nest boxes are rather small, with a diameter of 15 cm, and we never observed two separate nests within one box. We therefore assume that individuals have direct contact when simultaneously visiting a box. A total of 226 females provided 1305 monthly records of such data on partners, social interactions, and number of nest boxes used.

Adult females usually met for a rather extended period of time in a nest box. An average social interaction lasted 1296 s (21.6 min), and ranged between 2 s and 4602 s (76.7 min; Fig. 5.5). A short interaction, of less than 1 min, might also be interpreted as a socio-negative or agonistic encounter, resulting in a female being chased out of the box. Substantially longer interactions between females, however, suggest a socio-positive relationship or familiarity with each other, and that

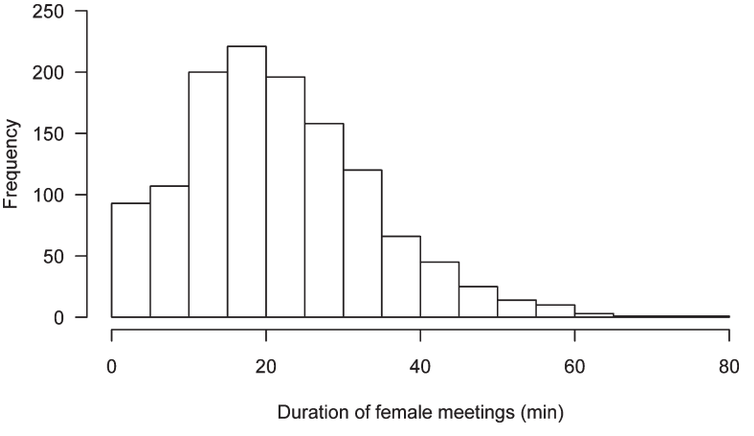

Figure 5.5 Frequency distribution of meeting duration (minutes) of any two tagged females that simultaneously visit a nest box (monthly average of the length of a female's social interactions with any other female; $N = 226$ females).

the females belong to the same social group. In 44 monthly records (5.7%), females used nest boxes without meeting another tagged female.

Influence of female reproductive state on spatial and social behaviour

In 615 monthly records, information was available on female reproductive status during that month from population-monitoring events, and was used for further analyses. We accounted for repeated measures of the same female by implementing linear mixed models in ASReml 3.0 (VSN International) with female identity as a random effect. We tested for independent and multiplicative effects of reproductive status and season on female social interactions. Accounting for repeated measures complicates determination of degrees of freedom; here, denominator degrees of freedom were calculated empirically in ASReml according to Kenward and Roger (1997).

Females differed substantially in the monthly number of nest boxes visited, which ranged between 1 and 33. Reproductively active females used fewer nest boxes than non-reproducing females (Fig. 5.6a). Furthermore, all females generally

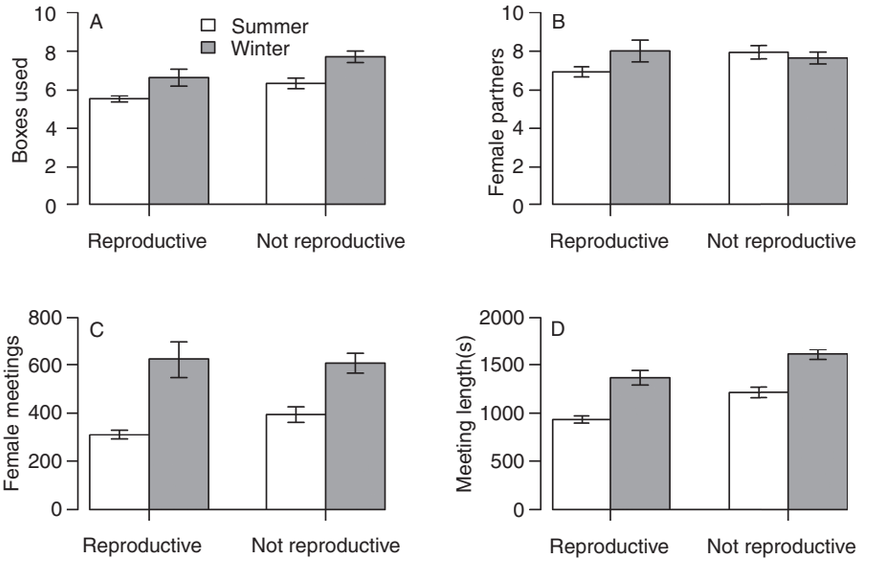

Figure 5.6 Bar plots (mean ± SE) of monthly data on (a) cumulative number of nest boxes visited; (b) cumulative number of female partners; (c) cumulative number of female meetings; and (d) average duration of meetings, according to female reproductive status and season (N = 615 monthly records).

used fewer boxes in summer than in winter (effect of reproduction: Wald statistic$_{1,551}$ = 20.0, p < 0.0001; season: Wald statistic$_{1,539}$ = 24.9, p < 0.0001; interaction: Wald statistic$_{1,545}$ = 0.001, p < 0.92). Within nest boxes, females met with 0–19 female partners, without obvious seasonal differences (Fig. 5.6b). Nevertheless, the lowest number of partners was observed for breeding females in summer (on average 6.9 partners), with significantly fewer partners than non-reproducing females in winter (reproduction: Wald statistic$_{1,488}$ = 3.38, p < 0.07; season: Wald statistic$_{1,490}$ = 0.38, p < 0.54; interaction: Wald statistic$_{1,491}$ = 8.38, p < 0.005).

Reproductively active females had fewer (Fig. 5.6c) and shorter meetings (Fig. 5.6d) than non-reproducing females, and during winter meetings generally were more frequent and longer than in summer (number of female meetings: reproduction: Wald statistic$_{1,541}$ = 12.2, p < 0.0001; season: Wald statistic$_{1,532}$ = 49.8, p < 0.0001; interaction: Wald statistic$_{1,537}$ = 2.26, p < 0.14; duration of female meetings: reproduction: Wald statistic$_{1,592}$ = 46.1, p < 0.0001; season: Wald statistic$_{1,569}$ = 49.9, p < 0.0001; interaction: Wald statistic$_{1,587}$ = 0.06, p < 0.81).

In both summer and winter females used only a limited number of nest boxes during a given month (on average 5–8 of 40 available), allowing for the conclusion that such spatial behaviour reflects an individual's home range. We have no information on female behaviour outside the nest boxes, but the rather long duration of the majority of meetings among adult females in a box suggests that partners belong to the same social group, are familiar with each other (with the potential to establish social bonds), and that all group members might contribute to defend nest boxes against non-group members (either through olfactory cues or via aggression during direct interactions; see also Hurst and Nevison, 1994).

Access to nest boxes may be a prerequisite for successful reproduction in females. The vast majority of litters in our population was born and weaned in boxes, presumably because they improve protection from disturbances by conspecifics (see also Crowcroft and Rowe, 1957, 1958; Hurst, 1987; Rusu *et al.*, 2004). Nevertheless, even reproducing females used several nest boxes and sometimes moved litters between boxes. We do not know why females regularly access several boxes even when lactating. Such behaviour can be explained by several factors, such as the option to move when faced with or disturbed by a predator, varying microclimatic needs during different environmental conditions, improved access to feeding and drinking sites, or avoidance of parasites. Since the number of partners significantly increased with the increasing number of nest boxes used (linear regression, $t_{1,1303}$ = 10.7, p < 0.0001), at a rate of 0.32 additional partners per additional box used, larger groups may be able to defend larger

territories, which again might improve access to resources and/or defence against non-group members.

Reproductive competition nevertheless influenced the spatial and social behaviour of the females. During summer, when a higher proportion of females were breeding, reproducing females used fewer boxes than during winter, and also fewer than non-reproducing females. When radio-tracking female house mice in a feed shed, Wilkinson and Baker (1988) also observed smaller home ranges in lactating females. Reproducing females might access fewer nest boxes because they regularly nurse offspring, keep them warm, and protect them against infanticidal conspecifics, and thus have less time for exploratory behaviour. On the other hand, they are limited in the time spent with the pups, because they have to drastically increase the daily amount of food eaten to allow for milk production (König *et al.*, 1988). Still, we exclude improved access to food as a causal factor to temporarily reduce home range size during breeding in summer. First, we did not observe food storage in nest boxes, and second, in winter, when nutritional and metabolic requirements are expected to be higher than in summer, reproducing females did not show reductions in home range size. Instead, we suggest that reproducing females under conditions suggesting high reproductive competition are more restrictive in access to social partners. As predicted from previous lab studies, they meet with relatively few partners, despite the fact that relatively more other females are breeding. Highly pregnant or lactating females might be more choosy in terms of with whom they meet, as preferences for social partners result in low conflicts over reproduction and improved offspring survival (see Weidt *et al.*, 2008). Furthermore, under experimental lab conditions individual lifetime reproductive success decreases with increasing number of females (König, 2006), and it has been shown for other rodents and larger mammals that female competition increases with increasing group size (reviewed in Silk, 2007).

Adult females rarely stayed in a nest box in the absence of any other adult female, suggesting that meetings in social groups and established social bonds play an important role in reproductive success. Social partners may serve various functions for a reproducing female. If the social partner is also lactating, both can establish a communal nest and gain mutual benefits through cooperative nursing of litters. During summer, on the other hand, relatively many non-reproducing adult females were also observed in our population. Social bonds to a non-reproducing female might allow a lactating female to gain benefits through helping to improve protection of offspring, social thermoregulation, or even allonursing by non-reproducing females.

Future analyses of fitness benefits arising from individual associations between reproducing and non-reproducing females in our study population will help us to

understand how the presence of other females influences individual reproductive success. If benefits of communal or cooperative care of young are substantial, we then predict that reproducing females are especially choosy with respect to social partners, and restrict interactions to only a few other reproducing females in order to maximize reproductive success (König, 1994a).

Presumably as a consequence of having fewer partners, reproducing females had fewer meetings during summer. In addition, they had shorter meetings in summer, the period of elevated reproductive competition. Such behaviour might suggest that breeding females alternated in their presence in the nest with other females, and minimized the time of simultaneous nest use. Alternating with other females reduces the time litters are left alone and are thus unprotected during periods of high energetic demand of the mother. Such a benefit would be obvious, especially for communally nursing females, and in captivity wild females have been observed to take turns nursing each other's offspring in communal nests (Wilkinson and Baker, 1988; König, 1989).

In winter, females had more partners, more meetings, and longer meetings than during summer, irrespective of their reproductive condition. Females thus decreased the time outside of nest boxes, presumably to minimize exposure to low temperatures in winter. In addition, larger groups may provide thermoregulatory benefits. Huddling as a social strategy against low temperature is widespread among rodents, and several otherwise solitary species nest communally during the winter, thereby reducing exposure to the environment and consequent heat loss (Martin *et al*., 1980; Batchelder *et al*., 1982; McShea, 1990; Hayes, 2000). The absence, or only weak presence, of reproductive competition during winter might have relaxed the reproducing females' selectivity in interactions with other group members, and thus allowed for the observed large number of partners. In African striped mice (*Rhabdomys pumilio*), a strictly seasonal breeder, reproductive competition even favours solitary living. After the breeding season, in the absence of reproductive competition, almost all striped mice live in groups even under very low population densities. During the breeding season, however, mice of both sexes may live solitarily, except under very high population density, when no opportunities for independent breeding exist (Schradin *et al*., 2010; 2012).

Conclusions

Our free-living study population of house mice in northern Switzerland showed distinct seasonality in female reproductive activity over a period of four years, despite unlimited access to food, water, and protected nesting sites. Such seasonality had interesting consequences for female reproductive competition and the quality of female–female interactions. During summer (April to

September), intrasexual competition among females was pronounced, and might explain why only a limited number of females were able to reproduce in the well-established population. Although population size and the number of adult females increased over the study period, the absolute number of reproductively active females remained unchanged. During winter (October to March), the proportion of reproducing females and the monthly number of offspring weaned were much lower, suggesting relaxed reproductive competition. It is unlikely that female house mice competed strongly over access to mates, since the number of adult males to adult females was rather equal over the entire period. They rather seemed to compete over resource availability, in particular access to nest boxes as a prerequisite to rear and protect a litter. Only when females drastically outnumber males or simultaneously share mate preferences, female competition may also encompass access to mates, as has been suggested by Rusu and Krackow (2004).

Throughout the year, females used a variety of nest boxes where they regularly interacted with several reproducing and adult non-reproducing female conspecifics belonging to the same social group. Despite the fact that analysis of long-term stability in interaction partners is still lacking, such a pattern underlines the significance of same-sex social partners and individualized social groups. Average life expectancy in the study population is 196 days, but adult females can live up to three or four years (Manser *et al.*, 2011). Very interestingly, generation time (average age of reproduction of a female), at nine months, is surprisingly long (Manser *et al.*, 2011), suggesting that especially younger females need to integrate and establish long-term social bonds within a group as a prerequisite to successfully wean offspring. Our long-term data thus propose that females maintain social bonds to other females even during periods of low reproductive competition, as part of a flexible and competitively superior reproductive strategy.

Within groups, social relationships appear to be structured by cooperation and by the existence and resolution of conflicts. During summer, when intrasexual competition was high, reproductively active females had relatively few partners and used few nest boxes. Limitation of lactating females' access to social partners is expected according to laboratory data, since individual reproductive success is highest for communally nursing pairs of females, and decreases with increasing group size (König, 2006). During winter, on the other hand, reproductive competition was low and the benefits of social thermoregulation might outweigh the benefits of reducing the number of social partners.

Theory predicts that the aversive effect of reproductive competition could be offset by kin association. Even when reproductive skew occurs within social groups, the long-term inclusive fitness of interacting females, when related, should then be higher than of females not exhibiting social preferences. Within social groups, female house mice have the option to establish preferences and

restrict meetings to fewer partners when reproducing under conditions of high intrasexual competition. Genetic relatedness among interacting partners has still to be analysed for our free-living population, as well as whether individual preferences result in fitness variances. Nevertheless, female social behaviour has to be interpreted in the context of long-term relationships and such relationships are typically complex. Interactions among females thus may be subject to social selection processes, driving the evolution of female traits. Future studies have to analyse whether females choose social partners based on their phenotype, to identify the traits they use for partner preferences, and whether social selection results in assortative traits of social partners.

REFERENCES

Alexander, R. D. (1974). The evolution of social behavior. *Annual Review of Ecology and Systematics*, **5**, 325–83.

Batchelder, P., Kinney, R. O., Demlow, L., and Lynch, C. B. (1982). Effects of temperature and social interactions on huddling behaviour in *Mus musculus*. *Physiology and Behavior*, **31**, 97–102.

Berry, R. J. (1968). The ecology of an island population of the house mouse. *Journal of Animal Ecology*, **37**, 445–70.

Berry, R. J. (1981). Town mouse, country mouse: adaptation and adaptability in *Mus domesticus* (*M. musculus domesticus*). *Mammal Review*, **11**, 91–136.

Bronson, F. H. (1979). The reproductive ecology of the house mouse. *The Quarterly Review of Biology*, **54**, 265–99.

Clutton-Brock, T. H. (2009). Sexual selection in females. *Animal Behaviour*, **77**, 3–11.

Crowcroft, P. (1966). *Mice All Over*. London: GT Foulis & Co.

Crowcroft, P. and Rowe, F. P. (1957). The growth of confined colonies of the wild house mouse (*Mus musculus* L.). *Proceedings of the Zoological Society of London*, **129**, 359–70.

Crowcroft, P. and Rowe, F. P. (1958). The growth of confined colonies of the wild house-mouse (*Mus musculus* L.): the effect of dispersal on female fecundity. *Proceedings of the Zoological Society of London*, **131**, 357–65.

Cucchi, T., Vigne, J.-D., and Auffray, J.-C. (2005). First occurrence of the house mouse (*Mus musculus domesticus* Schwarz Schwarz, 1943) in the Western Mediterranean: a zooarchaeological revision of subfossil occurrences. *Biological Journal of the Linnean Society*, **84**, 429–45.

Cucchi, T., Vigne, J.-D., Auffray, J.-C., Croft, P., and Peltenburg, E. (2002). Passive transport of the house mouse (*Mus musculus domesticus*) to Cyprus at the Early Preceramic Neolithic (late 9th and 8th millennia cal. BC). *Comptes Rendus Palevol*, **1**, 235–41.

D'Amato, F. R. (1997). Neurobiological and behavioral aspects of recognition in female mice. *Physiology & Behavior*, **62**, 1311–17.

D'Amato, F. R. and Moles, A. (2001). Ultrasonic vocalizations as an index of social memory in female mice. *Behavioral Neuroscience*, **115**, 834–40.

DeLong, K. T. (1967). Population ecology of feral house mice. *Ecology*, **48**, 611–34.

Dobson, F. W. and Baudoin, C. (2002). Experimental tests of spatial association and kinship in monogamous mice (*Mus spicilegus*) and polygynous mice (*Mus musculus domesticus*). *Canadian Journal of Zoology*, **80**, 980–6.

Dobson, F. W., Jacquot, C., and Baudoin, C. (2000). An experimental test of kin association in the house mouse. *Canadian Journal of Zoology*, **78**, 1806–12.

Drickamer, L. C. (1992). Oestrous female house mice discriminate dominant from subordinate males and sons of dominant from sons of subordinate males by odour cues. *Animal Behaviour*, **43**, 868–70.

Emlen, S. T. and Oring, L. W. (1977). Ecology, sexual selection, and evolution of mating systems. *Science*, **197**, 215–23.

Gray, S. J., Jensen, S. P. and Hurst, J. L. (2000). Structural complexity of territories: effects on preference, use of space and territorial defence in commensal house mice (*Mus domesticus*). *Animal Behaviour*, **60**, 765–72.

Hayashi, S. (1990). Social condition influences sexual attractiveness of dominant male mice. *Zoological Science*, **7**, 889–94.

Hayes, L. D. (2000). To nest communally or not to nest communally: a review of rodent communal nesting and nursing. *Animal Behaviour*, **59**, 677–88.

Hurst, J. L. (1987). Behavioural variation in wild house mice *Mus domesticus* Rutty: a quantitative assessment of female social organization. *Animal Behaviour*, **35**, 1846–57.

Hurst, J. L. (1990). Urine marking in populations of wild house mice *Mus domesticus* Rutty: II – communication between females. *Animal Behaviour*, **40**: 223–32.

Hurst, J. L. and Nevison, C. M. (1994). Do female house mice, *Mus domesticus*, regulate their exposure to reproductive priming pheromones? *Animal Behaviour*, **48**, 945–59.

Jennions, M. D. and Macdonald, D. W. (1994). Cooperative breeding in mammals. *Trends in Ecology and Evolution*, **9**, 89–93.

Kenward, M. G. and Roger, J. H. (1997). The precision of fixed effects estimates from restricted maximum likelihood. *Biometrics*, **53**, 983–97.

König, B. (1989). Behavioural ecology of kin recognition in house mice. *Ethology, Ecology Evolution*, **1**, 99–110.

König, B. (1993). Maternal investment of communally nursing female house mice (*Mus musculus domesticus*). *Behavioural Processes*, **30**, 611–74.

König, B. (1994a). Components of lifetime reproductive success in communally and solitarily nursing house mice: a laboratory study. *Behavioral Ecology and Sociobiology*, **34**, 275–83.

König, B. (1994b). Fitness effects of communal rearing in house mice: the role of relatedness and familiarity. *Animal Behaviour*, **48**, 1149–57.

König, B. (1997). Cooperative care of young in mammals. *Naturwissenschaften*, **84**, 95–104.

König, B. (2006). Non-offspring nursing in mammals: general implications from a case study on house mice. In *Cooperation in Primates and Humans: Mechanisms and Evolution*, ed. P. M. Kappeler and van C. P. Schaik. Berlin, Heidelberg: Springer-Verlag, pp. 191–205.

König, B. and Markl, H. (1987). Maternal care in house mice: I – the weaning strategy as a means for parental manipulation of offspring quality. *Behavioral Ecology and Sociobiology*, **20**, 1–9.

König, B., Riester, J., and Markl, H. (1988). Maternal care in house mice (*Mus musculus*): II – the energy cost of lactation as a function of litter size. *Journal of Zoology*, **216**, 195–210.

Latham, N. and Mason, G. (2004). From house mouse to mouse house: the behavioural biology of free-living *Mus musculus* and its implications in the laboratory. *Applied Animal Behaviour Science*, **86**, 261–89.

Laurie, E. M. O. (1946). The reproduction of the house mouse (*Mus musculus*) living in different environmental conditions. *Proceedings of the Royal Society B: Biological Sciences*, **133**, 248–81.

Lenington, S. and Egid, K. (1989). Environmental influences on the preferences of wild female house mice for males of differing *t*-complex genotypes. *Behavior Genetics*, **19**, 257–66.

Lewis, S. E. and Pusey, A. E. (1997). Factors influencing the occurrence of communal care in plural breeding mammals. In *Cooperative Breeding in Mammals*, ed. N. G. Solomon and J. A. French. Cambridge: Cambridge University Press, pp. 335–63.

Lidicker, W. Z., Jr (1976). Social behaviour and density regulation in house mice living in large enclosures. *Journal of Animal Ecology*, **45**, 677–97.

Lynch, C. B. (1992). Clinal variation in cold adaptation in *Mus domesticus*: verification of predictions from laboratory populations. *The American Naturalist*, **139**, 1219–36.

Manning, C. J., Dewsbury, D. A., Wakeland, E. K., and Potts, W. K. (1995). Communal nesting and communal nursing in house mice, *Mus musculus domesticus*. *Animal Behaviour*, **50**, 741–51.

Manser, A., Lindholm, A. K., König, B., and Bagheri, H. (2011). Polyandry and the decrease of a selfish genetic element in a wild house mouse population. *Evolution*, **65**, 2435–47.

Martin, R. A., Fiorentini, M., and Connors, F. (1980). Social facilitation of reduced oxygen consumption in *Mus musculus* and *Meriones unguiculatus*. *Comparative Biochemistry and Physiology*, **65A**, 519–22.

McShea, W. J. (1990). Social tolerance and proximate mechanisms of dispersal among winter groups of meadow voles, *Microtus pennsylvanicus*. *Animal Behaviour*, **39**, 346–51.

Nevison, C. M., Armstrong, S., Beynon, R. J., Humphries, R. E., and Hurst, J. L. (2003). The ownership signature in mouse scent marks is involatile. *Proceedings of the Royal Society B: Biological Sciences*, **270**, 1957–63.

Packer, C., Lewis, S., and Pusey, A. E. (1992). A comparative analysis of non-offspring nursing. *Animal Behaviour*, **43**, 265–82.

Palanza, P., Della Seta, D., Ferrari, P. F., and Parmigiani, S. (2005). Female competition in wild house mice depends upon timing of female/male settlement and kinship between females. *Animal Behaviour*, **69**, 1259–71.

Palanza, P., Re, L., Mainardi, D., Brain, P. F., and Parmigiani, S. (1996). Male and female competitive strategies of wild house mice pairs (*Mus musculus domesticus*) confronted with intruders of different sex and age in artificial territories. *Behaviour*, **133**, 863–82.

Pocock, M. J. O., Searle, J. B., and White, P. C. L. (2004). Adaptations of animals to commensal habitats: population dynamics of house mice *Mus musculus domesticus* on farms. *Journal of Animal Ecology*, **73**, 878–88.

Randall, J. A. (1999). *Vertebrate Pest Management: A Guide for Commercial Applicators*. East Lansing, MI: Michigan State University.

R Core Development Team (2011). *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.

Rosenqvist, G. and Berglund, A. (1992). Is female sexual behaviour a neglected topic? *Trends in Ecology and Evolution*, **7**, 174–6.

Rusu, A. S., König, B., and Krackow, S. (2004). Pre-reproductive alliance formation in female wild house mice (*Mus domesticus*): the effects of familiarity and age disparity. *Acta Ethologica*, **6**, 53–8.

Rusu, A. S. and Krackow, S. (2004). Kin-preferential cooperation, dominance-dependent reproductive skew, and competition for mates in communally nesting female house mice. *Behavioral Ecology and Sociobiology*, **56**, 298–305.

Sayler, A. and Salmon, M. (1971). An ethological analysis of communal nursing by the house mouse. *Behaviour*, **40**, 60–85.

Schradin, C., König B., and Pillay, N. (2010). Reproductive competition favours solitary living while ecological constraints impose group-living in African striped mice. *Journal of Animal Ecology*, **79**, 515–21.

Schradin, C., Lindholm, A. K., Johannesen, J., *et al*. (2012). Social flexibility and social evolution in mammals: a case study of the African striped mouse *(Rhabdomys pumilio). Molecular Ecology*, **21**, 541–53.

Silk, J. (2007). The adaptive value of sociality in mammalian groups. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **362**, 539–59.

Silk, J., Alberts, S. C., and Altmann, J. (2003). Social bonds of female baboons enhance infant survival. *Science*, **302**, 1231–4.

Singleton, G. R. (1983). The social and genetic structure of a natural colony of house mice, *Mus musculus*, at Healesville Wildlife Sanctuary. *Australian Journal of Zoology*, **31**, 155–66.

Solomon, N. G. and French, J. A. (1997). *Cooperative Breeding in Mammals*. Cambridge: Cambridge University Press.

Southwick, C. H. (1955). Regulatory mechanisms of house mouse populations: social behavior affecting litter survival. *Ecology*, **36**, 627–34.

Sterck, E. H. M., Watts, D. P., and van Schaik, A. P. (1997). The evolution of female social relationships in nonhuman primates. *Behavioral Ecology and Sociobiology*, **41**, 291–309.

Stockley, P. and Bro-Jørgensen, J. (2010). Female competition and its evolutionary consequences in mammals. *Biological Reviews*, **86**, 341–66.

Weidt, A., Hofmann, S. E., and König, B. (2008). Not only mate choice matters: fitness consequences of social partner choice in female house mice. *Animal Behaviour*, **75**, 801–8.

West-Eberhard, M. J. (1983). Sexual selection, social competition, and speciation. *The Quarterly Review of Biology*, **58**, 155–83.

West-Eberhard, M. J. (1987). Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology and Systematics*, **20**, 249–78.

Wilkinson, G. S. and Baker, A. E. M. (1988). Communal nesting among genetically similar house mice. *Ethology*, **77**, 103–14.

Yamazaki, K., Boyse, E. A., Miké, V., *et al*. (1976). Control of mating preferences in mice by genes in the major histocompatibility complex. *Journal of Experimental Medicine*, **144**, 1324–35.

Chapter 10

Mate choice for genetic compatibility in the house mouse

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Mate choice for genetic compatibility in the house mouse

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Abstract

In house mice, genetic compatibility is influenced by the *t* haplotype, a driving selfish genetic element with a recessive lethal allele, imposing fundamental costs on mate choice decisions. Here, we evaluate the cost of genetic incompatibility and its implication for mate choice in a wild house mice population. In laboratory reared mice, we detected no fertility (number of embryos) or fecundity (ability to conceive) costs of the *t*, and yet we found a high cost of genetic incompatibility: heterozygote crosses produced 40% smaller birth litter sizes because of prenatal mortality. Surprisingly, transmission of *t* in crosses using +/*t* males was influenced by female genotype, consistent with postcopulatory female choice for + sperm in +/*t* females. Analysis of paternity patterns in a wild population of house mice showed that +/*t* females were more likely than +/+ females to have offspring sired by +/+ males, and unlike +/+ females, paternity of their offspring was not influenced by +/*t* male frequency, further supporting mate choice for genetic compatibility. As the major histocompatibility complex (MHC) is physically linked to the *t*, we investigated whether females could potentially use variation at the MHC to identify male genotype at the sperm or individual level. A unique MHC haplotype is linked to the *t* haplotype. This MHC haplotype could allow the recognition of *t* and enable pre- and postcopulatory mate choice for genetic compatibility. Alternatively, the MHC itself could be the target of mate choice for genetic compatibility. We predict that mate choice for genetic compatibility will be difficult to find in many systems, as only weak fertilization biases were found despite an exceptionally high cost of genetic incompatibility.

Introduction

The question of why females choose mates in the absence of any direct benefits, such as access to resources, protection from harassment and predators, or provision of parental care, is still not resolved. Much theoretical and empirical research has addressed the "good genes" hypothesis of mate choice, where females base mate choice on the quality of genes that their offspring would inherit through the sire (indirect benefits) (Andersson and Simmons 2006). More recently, the indirect benefits of "compatible genes" have been investigated (Mays and Hill 2004). Under the "compatible genes" hypothesis, a female bases mate choice decisions on the potential interaction between the genes inherited through herself and her mate. The difference can be thought of as mate choice for a mate's breeding value (good genes) versus for a beneficial combination of the genes of the parents (Puurtinen et al. 2005). Genetic compatibility has the potential to influence offspring quality as much as beneficial genes of the sire, and thus may strongly influence mate choice evolution (Neff and Pitcher 2005; Puurtinen et al. 2009). Selection for genetic compatibility requires an individual to reference its own genotype through self-inspection or familiar imprinting, as well as those of potential mates, and to choose mates accordingly. Whereas genetic compatibility is at the heart of conspecific mate preference, its role in mate choice within populations is not clear. At the population level, genetic compatibility is likely to be limited to specific genetic systems, because complex interactions of male and female genotypes across many genes would place severe constraints on any such

Figure 1. Picture of house mice in the free-living study population (by Sabine Wunderlin).

system (Puurtinen et al. 2005). One such genetic system comprises the genes of the major histocompatibility complex (MHC), which are involved in immunocompetence and are believed to play an important role in mate choice (Jordan and Bruford 1998; Tregenza and Wedell 2000; Penn 2002; Milinski 2006; Yamazaki and Beauchamp 2007). It has been argued that genetic compatibility can only drive mate choice evolution within populations in two situations: inbreeding avoidance, in which the MHC has been implicated, and the coinheritance of compatibility and mate choice alleles (Tregenza and Wedell 2000).

Some of the strongest evidence for mate choice for genetic compatibility comes from the house mouse *Mus musculus* (Fig. 1) (Lenington and Coopersmith 1992), which carries a naturally occurring selfish genetic element, the *t* haplotype. The *t* haplotype evolved more than one million years ago and is composed of four linked inversions on chromosome 17 (Fig. 2) (Figueroa et al. 1985; Hammer et al. 1989), comprising a third of the chromosome (Silver 1993). The *t* haplotype contains at least one recessive lethal allele, and complementarity of recessive lethal alleles is used to define *t* haplotype variants (Artzt 1984). At least 16 different *t* haplotype variants are known (Klein et al. 1984). Heterozygotes of the same *t* haplotype variant produce *t*/*t* homozygotes that die before birth. However, heterozygotes for different *t* variants produce viable *t*/*t* offspring, but males are sterile (Dunn 1937). Moreover, the *t* haplotype shows drive (often called meiotic drive, segregation distortion, or transmission ratio distortion) in males (Chesley and Dunn 1936), which is the preferential transmission of one type of gamete to the next generation. Drive increases the proportion of offspring that inherit the *t*. To avoid *t*-related offspring mortality, heterozygous females should therefore prefer to mate with +/+ males. Such a preference would require tight linkage between the *t* and preference genes to avoid recombination breaking up the association (Price and Wedell 2008). Lenington and Egid proposed that a female preference gene lies within the *t* haplotype (Egid and Lenington 1985; Lenington and Egid 1985). They showed consistent odor preferences for +/+ males by +/*t* females and context-dependent preferences in +/+ females (Egid and Lenington 1985; Lenington and Egid 1985; Williams and Lenington 1993) in a series of experiments that have not been replicated elsewhere. It remains unclear if these laboratory-based odor preferences are generalizable to all *t* haplotype variants, and if they reflect actual fertilization bias when females mate in the wild, as female choice might be influenced by additional male quality traits (e.g., dominance status, MHC genotype, relatedness) and could be overridden by male mating behavior.

The *t* haplotype carries numerous MHC genes within its fourth inversion (Hammer et al. 1989). They code for cell receptors involved in presenting antigens to T-cells, thereby triggering specific immune responses against invading pathogens. Mice can smell the difference between congenic strains of laboratory mice that differ only at MHC alleles (Yamazaki et al. 1979), and in a

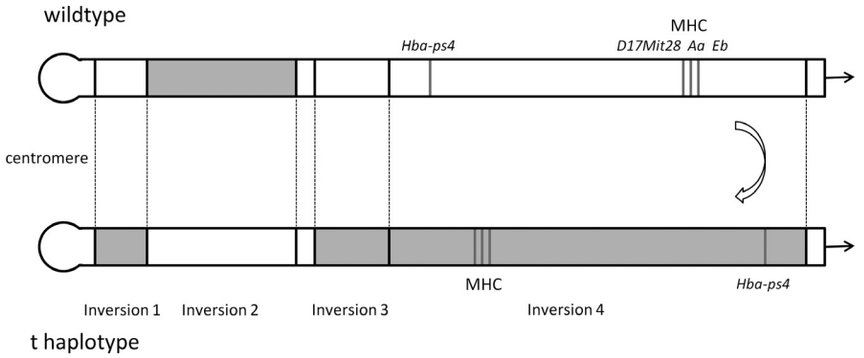

Figure 2. Schematic map of + and *t* haplotype forms of mouse chromosome 17. Shaded boxes represent the *t*-associated inversions. The MHC markers genotyped here are indicated. The *Hba-ps4* marker was used for *t*-haplotype identification.

laboratory setting, they choose mates according to MHC type (reviewed in Yamazaki and Beauchamp 2007). However, the extent to which MHC is used in individual recognition and mate choice in house mice is controversial (see Cheetham et al. 2007; Sherborne et al. 2007). The MHC could also provide a marker enabling postcopulatory (cryptic) female choice, via egg-sperm interactions (Wedekind et al. 1996), possibly modulated via MHC-linked olfactory receptor genes (Ziegler et al. 2002). If the *t* haplotype is associated with unique, but phylogenetically related, alleles at the MHC (e.g., Figueroa et al. 1985; Ben-Shlomo et al. 2007), MHC-mediated signals could provide the potential for female mice to discriminate between *t* and +.

In this study, we use a wild house mouse population and its laboratory-born descendants to address three aspects of mate choice for genetic compatibility. First, we use laboratory-born mice to estimate drive and empirically measure the cost of mating with a genetically incompatible mate in terms of litter size reduction. Costs to females could be low through overproduction of zygotes, leading to a litter size similar to that of genetically compatible crosses, despite prenatal mortality of *t*/*t* homozygotes (Charlesworth 1994), following from the selection arena hypothesis (Stearns 1987). Second, we use genetic paternity analysis to investigate whether wild females show a fertilization bias according to genetic compatibility. Finally, we address a mechanism that could allow females to discriminate between males on the basis of the *t* haplotype. We assess variation in the functionally important antigen binding sites of two MHC loci and a linked microsatellite to evaluate whether the *t* haplotype is associated with a unique set of MHC alleles.

Methods

Study species

Wild house mice (*Mus musculus domesticus*) were studied in a 72 m^2 farm building near Illnau, Switzerland. This population was founded in November 2002 by 12 mice (four males, eight nonpregnant females) caught in the local area, from the two nearest known natural populations. Unknown to us at the time, mice from one of the populations were all +/+, while four of six mice released from the second population were +/*t*. The concrete floor was covered with sawdust and straw, 40 PVC nest boxes, and plastic tubes, bricks, and branches for use as hiding places. Vertical metal plates with holes to allow the passage of mice provided further substructure. Food (grains, oat flakes, and rodent pellets) and water were provided ad libitum at feeding and water stations. These conditions are similar to those found in natural house mouse populations, as house mice in Europe live commensally with humans and typically occur where food is plentiful, such as in stables or granaries (Berry et al. 2008). While the building was permeable to house mice through numerous small openings, larger predatory animals, such as cats, foxes, and owls were excluded, although these animals did occur outside the barn. For further details, see König and Lindholm (2012). All research described here received ethics approval and was conducted in accordance with Swiss law.

t haplotype diagnosis

We identified *t* haplotype status on the basis of genetic tests of tissue samples (ear punches, and in some cases from the tail of deceased animals). DNA isolation was performed by salt-chloroform extraction (Müllenbach et al. 1989). For each animal we amplified the genotype at the *Hba-ps4* (alpha-globin pseudogene-4) locus, which occurs in the fourth inversion of the *t* haplotype, using the primers Hb.1 and Hb.2 (Schimenti and Hammer 1990). Compared to the + allele, the *t* allele at the *Hba-ps4* locus contains an insertion of 16 nucleotides, and this size difference is easily scored using a 3730xl DNA Analyzer (Applied Biosystems, Zug, Switzerland) and Genemapper software (Applied Biosystems). This method of scoring the *t* haplotype has been reliably used in previous studies (Schimenti and Hammer 1990; Huang et al. 2001; Carroll et al. 2004; Manser et al. 2011).

Cost of genetic incompatibility

The cost of genetic incompatibility was assessed by comparing the number of offspring in laboratory crosses between virgin +/*t* females mated to +/*t* males or to +/+ males. As controls, we included the mating crosses of virgin +/+ females with +/*t* or +/+ males. This experiment was performed in an animal breeding facility of the University of Zurich under standard laboratory conditions in 2009. We used F1 to F3 descendants of wild-caught house mice from our Illnau study population caught between 2006 and 2008. All individuals were tissue sampled and genotyped at the *Hba-ps4* locus, as described above.

In the first experiment, we compared litter sizes at birth in 53 crosses between all combinations of +/+ and +/*t* males and females, avoiding sib–sib matings. An adult male and an adult female were placed together into a clean cage (Macrolon type III, 425 × 266 × 155 mm) with bedding (Lignocel® Bedding, London, U.K.) and nesting material (paper and cardboard). Food pellets (mouse and rat breeding diet from Provimi Kliba AG, Kaiseraugst, Switzerland) and water were provided ad

libitum. After 14 days, males were removed. From 18 days postmating, females were checked daily for birth. If pups were found, or a female was no longer heavily pregnant, we searched the cage to locate all living pups as well as any that died after birth.

To investigate whether litter size differences were the result of genetic incompatibility leading to embryonic mortality, we conducted a second experiment using 74 crosses between all combinations of +/+ and +/*t* males and females, again avoiding sib–sib matings. Here, we estimated litter sizes at birth as well as prenatal mortality by examining placental scars in the mother's uterus shortly after birth. On the day of birth of the litter we euthanized the mother and her pups. Under a dissecting microscope, the uterus of each female was dissected and examined for uterine scars, as in Chesley and Dunn (1936). Two types of uterine marks were scored (see Fig. 2 of Krackow 1992). One type consisted of bloody markings in the middle of rosettes of tissue, referred to as "red" scars (Krackow 1990). The other type of mark consisted of yellow swellings, or "yellow" scars. Red scars indicate the implantation sites of pups which were just born, while yellow scars mark embryos that died after implantation and were resorbed (Krackow 1990, 1992). In this way we could estimate how many implanted embryos were viable versus inviable. One female was found to have only one functional uterine horn; she was then removed from the dataset. Data were analyzed in R 2.12.2 (R Development Core Team 2011), using a generalized linear model (GLM) in the MASS package (Venables and Ripley 2002) using litters as the unit of analysis and binomial errors. Contrasts were used for significance tests of differences between crosses. Pup survival from birth until weaning in the first experiment was analyzed similarly. Data were not overdispersed.

Estimating drive

We estimated drive by examining the inheritance of the *t* haplotype in the breeding crosses above. The unit of analysis was the litter of a female, and the response variable was a vector comprised of the numbers of +/*t* and +/+ offspring in her litter. GLM modelling including confidence interval estimation was carried out using quasibinomial errors, using the MASS package in R 2.12.0. Our expected value for the proportion of +/*t* offspring at birth in heterozygote crosses was the expected number of +/*t* offspring divided by the expected number of +/*t* plus +/+ offspring or $(0.5/(0.5 + [1 - \mathrm{TRD}]/2))$ (Manser et al. 2011), where TRD is the transmission ratio distortion estimated from crosses of +/*t* males mated to +/+ females.

Mate choice for genetic compatibility in a wild population

Genetic parentage analysis

We performed a parentage analysis of our wild study population. The population was monitored regularly from November 2002 until February 2005 and from August 2005 until December 2005. For this study, we used all pups born ($N = 201$) between 27 July 2004 until 20 December 2005, as the male population was then relatively large, providing many males for females to choose between.

Nest boxes were checked every week for the presence of litters. Ear punches for genetic analyses were taken from pups at the approximate age of 13 days, before pups begin to be mobile. All mice were regularly captured (approximately once a month), sexed, and weighed. As adults, mice were again ear punched and implanted with Trovan transponders for individual identification using a portable transponder reader (LID 500 Hand-Held Reader, TROVAN electronic identification systems, Weilerswist, Germany). Dates of death of transpondered mice were recorded, and tissue samples for genetic analyses were taken from all untranspondered corpses that were found at the study site.

For parentage and identity analyses we used 21 microsatellite loci spread across 17 autosomes excluding chromosome 17, which carries the *t* haplotype. The loci (Chr1_20, D2Mit145, D3Mit278, D4Mit227, Chr5_20, D5Mit122, D6Mit139, D6Mit390, D7Mit17, D7Mit319, Chr8_3, D9Mit201, Chr10_11, D11Mit150, D11Mit90, Chr12_2, D13Mit88, D14Mit44, D16Mit139, D18Mit194, and Chr19_17) were amplified together with the *Hba-ps4* marker for *t* haplotype diagnosis, and a MHC-linked microsatellite (D17Mit28) in four multiplex polymerase chain reactions (PCRs). Marker details are available elsewhere (Meagher and Potts 1997; Bult et al. 2008; Teschke et al. 2008). PCR reactions used the Qiagen Multiplex PCR Kit or AmpliTaq Gold DNA Polymerase (Applied Biosystems) and a final concentration of 0.075–0.4 μmol/L primer for 28–31 cycles at an annealing temperature of 60°C. Negative and positive controls were included on each plate. PCR products were analyzed using a 3730xl DNA Analyzer and Genemapper software.

To test if variation at these 21 autosomal markers met expectations for neutral markers, we used a Hardy–Weinberg (HW) test as implemented in Genepop on the Web version 4.0.10 (Raymond and Rousset 1995; Rousset 2008), using a sample of all adult and subadult mice (25 females, 31 males) that were present in the barn in July and August 2004. There was no significant deviation from HW equilibrium in a global test across loci using Fisher's method ($\chi^2 = 50.89$, df = 42, $P = 0.163$).

Parentage analyses of the pup-mother-father trio were performed for 2004 and 2005 using CERVUS 3.0 (Kalinowski et al. 2007). Behavioral assignment of maternity is not possible in this population due to communal nesting. Parentage assignments were accepted at a confidence level of 95% with two or fewer mismatches between the mother–father–offspring trio. We considered as candidate parents all adult mice detected alive in the barn at least once within 30 days prior to the estimated birth date of a pup. While gestation in house mice lasts ca. 19 days (Theiler 1989), our 30 day period is conservative, but accounts for the possibility that individuals may be missed during individual monitoring sessions, and for variability in monitoring intensity. The genotyping error rate for CERVUS was determined by repeated PCR amplification and genotyping of 100 individuals, on average, per locus, for a total of 3837 alleles scored. This gave an error rate (frequency of alleles scored differently between PCR amplifications) of 0.006. An error rate of 0.01 was used in CERVUS analyses. In 2004, as monitoring was intense, we estimated the proportion of sampled mothers and fathers at 90%. The average number of candidate mothers per offspring was 21 and of candidate fathers was 24. The proportion of loci typed was 0.99. In 2005, with less frequent monitoring, we estimated the proportion of sampled mothers and fathers at 75%. The average number of candidate mothers per offspring was 42 and candidate fathers was 25. The proportion of loci typed was 0.98. Offspring without an assigned male and female parent were excluded from further analysis. Multiple paternity was scored when more than one sire was assigned to pups within a litter. The confidence interval around the multiple paternity estimate was calculated by bootstrapping the dataset, following the method of Eccard and Wolf (2009), which takes litter size into account.

Tests of the effect of genotype on paternity

Effect of female genotype at the *t* haplotype on paternity of offspring was assessed in several ways. In an initial analysis, we compared the proportion of offspring born to +/*t* and +/+ females sired by each genotype using Pearson χ^2 tests. We then refined the dataset to singly sired litters to test female choice of sire for her litter, using Pearson χ^2 tests. To allow for multiple paternity within a litter, we used a GLM approach. Two analyses were performed in R 2.12.0, both specifying a binomial error distribution. In the first, using the lme4 package (Bates et al. 2011), we used each litter as the unit of analysis. The response variable was comprised of two vectors: for each litter, the number of offspring sired by any +/*t* male and the number of offspring sired by any +/+ male. We used female genotype, proportion of +/*t* males present in the month before birth, their interaction, and year as fixed effects. Female identity was included as a random effect to account for repeated measures. The data appeared overdispersed. As a quasibinomial analysis using GLMM is no longer supported in lme4 (see http://cran.r-project.org/web/packages/lme4/ChangeLog), the significance of female genotype was (approximately) assessed by comparing deviance values of nested maximum likelihood models, including and excluding a predictor variable. Differences in deviance approximate a chi-squared distribution with one degree of freedom. We then considered a model in which each pup was considered an independent fertilization. Paternal genotype was the response variable, and we used the same fixed effects as above in a GLM analysis.

All analyses were performed using observed paternities, and where relevant, after applying a correction factor in the cases where a +/*t* female produced offspring from a +/*t* male. When such crosses occur, offspring of the *t*/*t* genotype will die prenatally and will not be sampled, thus underestimating paternity from +/*t* males. We accounted for this bias by multiplying the observed number of offspring per litter from +/*t* females and +/*t* males by the percentage of litter size reduction we observed in the laboratory in such crosses, and adding these as "virtual" pups to that litter. This had the effect of increasing the number of pups of +/*t* females sired by +/*t* males.

We also estimated drive from litters that were sired by males of a single genotype, analyzing them in the same way as the laboratory crosses (see above).

MHC genotyping

We genotyped 29 mice from the barn population (15 +/+ and 14 +/*t* haplotype carriers, including all founder mice and a random sample of the population) at two MHC class II loci, Aα and Eβ on chromosome 17 (see Fig. 2) using single-stranded conformation polymorphism (SSCP). We amplified a fragment of exon 2 (antigen binding site) for both loci using the following primers: Aα-F: 5′-ACC ATTGGTAGCTGGGGTG-3′ and Aα-R: 5′-CTAAATCC ATCAGCCGACC-3′ for Aα (226 bp); JS1 5′-GAGTGTCA TTTCTACAACGGGACG-3′ and JS2 5′-GATCTCATAGT TGTGTCTGCA-3′ for Eβ (171 bp) (modified after Schad et al. 2004). Ten microliter reactions contained 0.5–1 μL of extracted genomic DNA, 1 Ml 10× Reaction buffer B (Solis BioDyne, Tartu, Estonia), 0.2 mmol/L dNTPs, 1.5 mmol/L $MgCl_2$, 1U FIREPol® DNA Polymerase (Solis BioDyne) and 0.3 μmol/L of each primer for Aα, respectively, 0.5 μmol/L for Eβ. Cycling conditions consisted of an initial denaturation at 94°C for 2 min followed by 10 rounds of 30 sec denaturation at 94°C, 30 sec annealing at 59°C (Aα)/53°C (Eβ), and 60 sec extension at 72°C,

followed by 25 rounds of denaturation at 94°C for 30 sec, annealing at 54°C (Aα)/48°C (Eβ) for 30 sec, 72°C extension for 60 sec. A final 10 min extension at 72°C followed the last cycle. For SSCP analyses, 1 μL of diluted PCR product (dilution Aα 1:60; Eβ 1:50) was combined with 14 μL loading dye mix (13.75 μL Hi-Di™ formamide, 0.25 μL GeneScan™ 350 ROX™ size standard [Applied Biosystems]). The mixture was denatured for 6 min at 95°C, immediately chilled on ice for 2 min and analyzed by capillary electrophoresis on an ABI PRISM® 3130xl automated DNA Sequencer (Applied Biosystems). The CE-SSCP polymer consisted of 5% conformational analysis polymer (CAP: made of 9% CAP, 10× genetic analyze buffer, 100% glycerol, and HPLC-water) and a 1× ABI running buffer was used. Separation of allelic variants was achieved by using the following run conditions: injection voltage at 1.2 kV, injection time of 18 sec, run voltage at 12 kV for 40 min, run temperature at 22°C. The retention times of the allelic variants were identified relative to the 350 ROX™ size standard using GeneMapper software.

Alleles were confirmed by direct sequencing of PCR products of ≥ 2 (preferentially) homozygote individuals following the manufacturer's instructions (Applied Biosystems). Sequences were edited and compiled with BioEdit 7.1.3.0 (Hall 1999). Sites involved in antigen binding were identified (Brown et al. 1993; Reche and Reinherz 2003). Microsatellite data obtained by paternity analyses (21 neutral markers) and one MHC-linked microsatellite (D17Mit28; Meagher and Potts 1997) were included for comparison of different selection patterns. We used the program Genepop (Raymond and Rousset 1995; Rousset 2008) to test for population differentiation and deviations from HW expectation.

Results

Cost of genetic incompatibility

The cost of genetic incompatibility is the reduction in litter size that a +/*t* incurs when mating with a +/*t* rather than a +/+. In experiment 1, we estimated the cost of genetic incompatibility to females by performing all possible crosses of +/*t* and +/+ genotypes and counting litter size at birth. Litter size differed between crosses (Table S1; ANOVA, $F_{3,49} = 4.04$, $P = 0.012$); +/*t* females mated to +/*t* males produced litters significantly smaller than those of all other crosses (for all contrasts, $t_{1,49} > 2.34$, $P < 0.023$). Litter sizes at birth may have been underestimated, as we found remains of dead pups in five instances, implicating infanticide, with a further suspicion of such causes in two more cases. To rule out maternal cannibalism, we conducted a second experiment, examining the uteri of females shortly after giving birth.

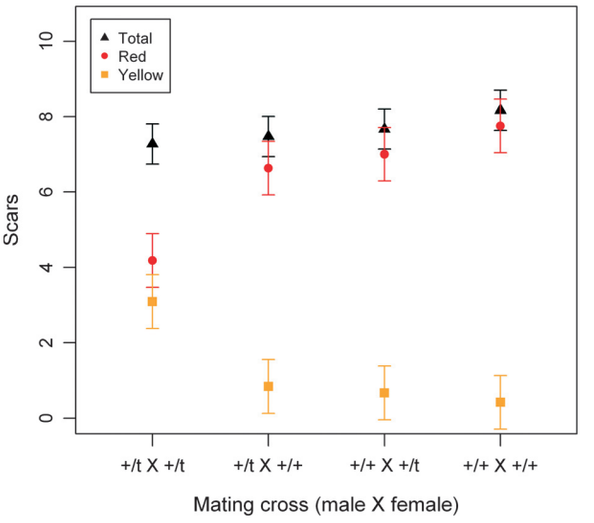

Figure 3. Number of uterine scars ± 95% CI per mating cross. Red scars indicate live births and yellow scars prenatal mortality.

In experiment 2, we conducted additional crosses to compare fertility, measured as the total number of uterine scars, which indicates the number of embryos implanted in the uterus. Fertility did not differ significantly among crosses (Fig. 3, ANOVA, $F_{3,70} = 1.40$, $P = 0.249$). The overall mean number of uterine scars per female was 7.58 (±0.15 SE). This indicates the average litter size at birth that would have resulted from the survival of all implanted embryos, regardless of genotype. Counts of red scars differed among crosses (Fig. 3, ANOVA, $F_{3,70} = 16.12$, $P = 0.001$). Red scars indicated that +/*t* × +/*t* matings resulted in a mean of 4.18 (±0.42 SE) offspring, significantly fewer than that detected in all other groups ($t_{1,70} > 4.67$ for all contrasts, $P < 0.001$), on average a loss of 3.40 pups per litter. Litter size at birth was highly correlated with the number of red scars (Pearson correlation, $N = 74$, $r = 0.92$, $P < 0.001$). Mean litter size at birth (Table S1) also differed significantly among the four types of crosses (ANOVA, $F_{3,70} = 14.67$, $P < 0.001$). While yellow scars were found in females of all mating crosses (Fig. 3), indicating prenatal embryonic mortality, there were significant differences between mating crosses ($F_{3,70} = 16.73$, $P < 0.001$). More yellow scars were found in +/*t* × +/*t* crosses, averaging 3.09 (±0.37 SE) (Fig. 3; $t > 5.31$ for all comparisons, $P < 0.001$). Compared with all other crosses combined, which averaged 0.67 (±0.16 SE), an excess of 2.42 yellow scars was found in +/*t* × +/*t* matings.

Overall, 79.3% of mating crosses were fecund (yielded offspring). Fecundity, however, did not differ according to type of mating cross ($\chi^2_{3,127} = 1.85$, $P = 0.603$). We also tested for a possible advantage to +/*t* females mated

to +/*t* males – with a smaller litter size, they might give birth sooner. However, neither litter size nor mating cross predicted time to birth ($F_{4,119} = 0.35$, $P = 0.842$). Furthermore, using data from experiment 1, we tested for a difference in pup survival until weaning from different crosses and found no difference among crosses (binomial GLM, $\chi^2_{3,43} = 0.16$, $P = 0.999$).

The cost of genetic incompatibility to +/*t* females can be calculated by comparison of litter sizes at birth, of the number of red scars, or of yellow scars. Counting litter sizes after birth gave an estimation of reduction in litter size of 40.8% (combining experiments 1 and 2; 95% CI 30.6–50.8). Comparing red scars, which is a better indicator of the number of pups to which a female gave birth, the reduction is similar at 40.3%. Finally, from the excess of yellow scars in +/*t* × +/*t* matings compared to the average for all other matings, the reduction in litter size is estimated to be 32.0%. From the male +/*t* point of view, the cost of mating with a +/*t* female compared with a +/+ female was a litter size loss of 37.5% (combining experiments 1 and 2; 95% CI 26.8–48.2). From comparison of red scars, the reduction was 36.9%, while the estimate based on yellow scars is the same as for females.

Drive

Pups from the laboratory crosses were genotyped to estimate the degree of drive associated with the *t* haplotype. As expected, no pups were homozygous for *t*. Transmission ratios varied with the type of mating cross (GLM, $\chi^2_{2,91} = 72.15$, $P < 0.001$; Fig. 4). When +/*t* males and +/+ females were crossed, 89.7% of 175 offspring from 30 litters inherited the *t*. In crosses between +/*t* males and +/*t* females, 78.6% of 109 offspring that were born in 32 litters inherited the *t* haplotype, a significantly lower proportion than in the previous cross (GLM, $t = 2.18$, $P = 0.032$). In the reciprocal cross of +/+ male and +/*t* female, 51.8% of 203 offspring from 32 litters inherited the *t* haplotype, which was not different from 0.5 (exact binomial test, $P = 0.674$).

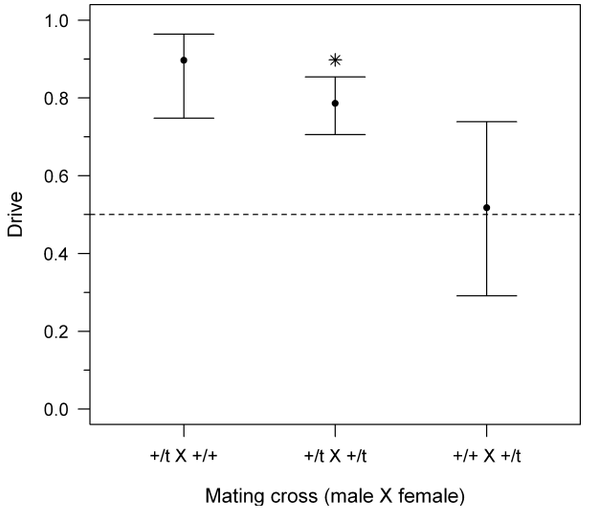

Figure 4. Drive estimates ± 95% CI for each mating cross. The expected value (asterisk) for crosses of +/*t* males and +/*t* females lies outside the observed value. The dashed line indicates the Mendelian expectation of 0.5.

We further investigated the difference in transmission rate of the *t* haplotype inherited through the male, depending on the female genetic background. Based on 89.7% transmission to offspring in +/*t* male and +/+ female crosses, and 50% transmission in crosses of +/+ male and +/*t* females, the proportion of +/*t* offspring in crosses of +/*t* males and +/*t* females was expected to be 90.7%, taking into account *t*/*t* lethality. The 95% confidence interval around the estimate of the observed value (78.6%) does not overlap this expected value (Fig. 4).

Mate choice for genetic compatibility in a wild population

Genetic parentage analyses and proportion of +/*t* males in the population

Both mother and father could be identified by genetic parentage analysis to 186/201 offspring at a confidence level of 95% (2004: 144/146 offspring; 2005: 42/55 offspring). Offspring where both parents could not be unambiguously identified were excluded from analysis: in 2004, two offspring could not be assigned fathers whereas in 2005, 11 offspring could not be assigned mothers and two additional offspring could not be assigned fathers.

Frequency of the *t* among candidate parents at the time of putative mating, according to litter birth date, is shown in Figure 5. The proportion of males present at the time of putative mating that were of +/*t* genotype differed between 2004 and 2005 (Wilcoxon rank sum test, $W = 144.50$, $P < 0.001$), with an average proportion of 0.55 (±0.01 SE) in 2004 and 0.65 (±0.01 SE) in 2005. We therefore included a year effect in our analyses. These data are not truly independent, however, as individuals differed in tenure in the population. To test whether the actual proportions of +/*t* and +/+ males differed overall, we compared the genotypes of those individuals detected in the population (Table 1). Among the potential sires, the proportion of +/*t* did not differ from 0.5 (exact binomial test: 2004, 0.53 were +/*t*, $P = 0.780$; 2005, 0.59 were +/*t*, $P = 0.200$). Among potential mothers, the proportion that were +/*t* (0.63) differed from 0.5 in 2005 (Table 1; $P = 0.023$), but not in 2004 ($P = 0.243$). Although a higher proportion of +/*t* females (0.46) produced offspring in

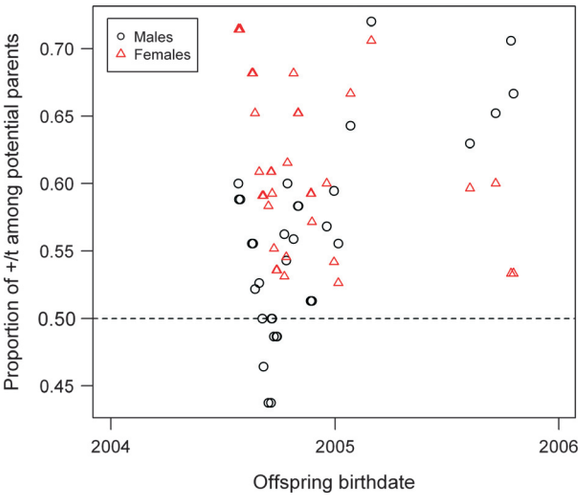

Figure 5. Proportion of +/*t* adult males and females according to litter birth date. The dashed line indicates a 1:1 proportion of +/*t* to +/+.

2004 than did +/+ females (0.26), the difference was not significant (Pearson's $\chi^2 = 1.18$, df = 1, $P = 0.277$).

Analysis of paternity patterns in relation to +/*t* genotype

Overall, 33.8% of pups of +/*t* females were sired by +/*t* males, compared to 63.8% of pups of +/+ females (Table 1), a highly significant difference (Pearson's $\chi^2 = 11.83$, df = 1, $P < 0.001$). However, the number of offspring sired by +/*t* males is likely to be underestimated for +/*t* females because of 40.3% prenatal mortality, our best estimate from the laboratory crosses (as *t*/*t* offspring die). If we correct for this, the estimate of proportion of offspring of +/*t* females sired by +/*t* males rises to 46.2%, which is still different from the proportion of offspring of +/+ females sired by +/*t* males ($\chi^2 = 3.91$, df = 1, $P = 0.048$). Multiple paternity in litters of two or more pups occurred in 32.5% (95% CI: 17.5–47.5) of litters overall. For +/*t* females, multiple paternity was observed in 9/24 litters in 2004 and 0/6 litters in 2005, or 30% overall (95% CI: 13.3–46.7). In four cases, paternity of the litter was divided between +/*t* and +/+ males. For +/+ females, 3/4 litters of two or more pups had multiple sires in 2004, and 1/6 in 2005, for a total of 40% (95% CI: 10.0–70.0). In two cases the paternity of the litter was shared by +/*t* and +/+ males.

Multiple paternity complicates analysis of female choice. We considered the simplest case, when a single male sired all offspring (i.e., no multiple paternity occurred), and asked if female genotype had an effect on her choice of sire. In 2004, 9/24 litters of +/*t* females and 2/4 litters of +/+ females were sired by +/*t* males. In 2005, 0/9 litters of +/*t* females and 4/6 litters of +/+ females were sired by +/*t* males. Overall, there was a significant effect of female genotype on paternity of the litter (Pearson's $\chi^2 = 12.31$, df = 4, $P = 0.015$). Data from 2005 alone showed a significant difference according to female genotype ($\chi^2 = 5.13$, df = 1, $P = 0.023$) but data from 2004 did not ($\chi^2 = 0.01$, df = 1, $P = 0.937$).

We then incorporated the possibility of having multiple sires within a litter into the analysis using a mixed-effect GLM with the proportion of pups in the litter that were sired by a male of +/*t* genotype, weighted by litter size, as the response variable. Predictor variables were female genotype, proportion of +/*t* males present before birth of the offspring, their interaction, and year. The dataset consisted of 56 litters from 29 females; therefore, we used maternal identity as a random effect to account for multiple litters from the same female. The interaction of female genotype and proportion of +/*t* males was significant (log-likelihood ratio test, $\chi^2 = 9.56$, df = 1, $P = 0.002$), as was the effect of year ($\chi^2 = 27.70$, df = 1, $P < 0.001$). Given the significant interaction term, the main effects marginal to it could not be tested independently (Fox 1997). If the interaction term

Table 1. Details of genetic parentage analyses.

Genotype	*N* breeding females	*N* nonbreeding females	*N* breeding males	*N* nonbreeding males	*N* offspring	*N* offspring sired by +/*t*	Proportion of offspring sired by +/*t*	Lower SE–Upper SE	Corrected for *t*/*t* mortality
2004									
+/*t*	13	15	13	14	118	47	0.398	0.354–0.444	0.527
+/+	5	14	12	12	26	16	0.615	0.570–0.659	0.615
2005									
+/*t*	7	47	5	31	21	0	0	0–1	0
+/+	7	25	6	19	21	14	0.667	0–1	0.667
Combined									
+/*t*	19	49	17	25	139	47	0.338	0.299–0.379	0.462
+/+	10	31	16	16	47	30	0.638	0.595–0.678	0.638

was dropped from the model, then both female genotype ($\chi^2 = 4.65$, df = 1, $P = 0.031$) and proportion of +/*t* males ($\chi^2 = 13.88$, df = 1, $P < 0.001$) were significant. When data were corrected for *t*/*t* mortality, *P* values were yet smaller (not shown).

To further explore the interaction between female genotype and proportion of +/*t* males on sire paternity, we used a binary GLM with paternity of each pup as the unit of analysis. This assumes that paternity of pups within a litter is independent, which is not unreasonable as our data showed multiple paternity within litters. There was a significant interaction of female genotype with the proportion of +/*t* males in the population ($\chi^2 = 15.85$, df = 1, $P < 0.001$). +/+ females were more likely to have their offspring sired by +/*t* males when there were many +/*t* males available, while this had no influence on +/*t* females (Fig. 6). Female genotype was a significant predictor of sire genotype ($\chi^2 = 12.94$, df = 1, $P < 0.001$), while the proportion of +/*t* males in the population did not have a significant effect ($\chi^2 = 0.00$, df = 1, $P = 0.961$). Year also had a significant effect ($\chi^2 = 14.55$, df = 1, $P < 0.001$). Correcting for *t*/*t* mortality still gave significant (all $P < 0.050$) results for female genotype, its interaction with the proportion of +/*t* males, and year.

Drive in the wild population

The degree of transmission bias of the *t* in litters from the wild population sired by a single genotype was estimated, and analyzed in a binary GLM, using litters as the main unit of analysis. The overall model was significant

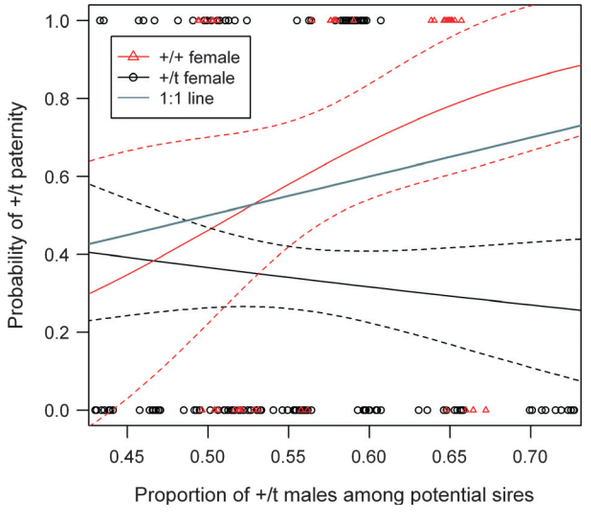

Figure 6. Incidence function of paternity by a +/*t* male relative to the proportion of +/*t* males among potential sires and to female genotype, with 95% confidence intervals. The gray line indicates a 1:1 relationship.

Table 2. Allele frequency and heterozygosity of MHC loci among 14 +/*t* and 15 +/+ mice.

	Locus					
	D17Mit28		Aα		Eβ	
Allele	+/+	+/*t*	+/+	+/*t*	+/+	+/*t*
1 (*t*)	**0.000**	**0.500**	**0.000**	**0.500**	**0.000**	**0.500**
2	0.233	0.036	0.167	0.036	0.067	0.107
3	0.067	0.107	0.067	0.000	0.233	0.036
4	0.367	0.250	0.768	0.464	0.700	0.357
5	0.333	0.107				
H_{Obs}	0.400	1.000	0.267	1.000	0.200	1.000
H_{Exp}	0.720	0.688	0.393	0.553	0.467	0.632
Exact tests						
P	<0.001		<0.001		<0.001	

Alleles are arbitrarily numbered; in bold are *t*-specific alleles. Exact tests for population differentiation are given (Raymond and Rousset 1995).

($F_{2,42} = 17.45$, $P = 0.003$), which was due to a difference between crosses of +/*t* males mated with +/+ females, in which 24/28 (85.7%) offspring from seven litters inherited the *t*, compared to crosses of +/+ males mated with +/*t* females, in which 33/76 (43.4%) offspring from 25 litters inherited the *t* ($t_{1,42} = 3.03$, $P = 0.004$). The proportion of +/*t* offspring detected in these crosses did not differ from that of crosses of +/*t* males mated with +/*t* females, in which 26/40 (65.0%) of offspring in 13 litters inherited the *t* (GLM, $t_{1,42} = 1.60$, $P = 0.117$ for the former and $t_{1,42} = -1.89$, $P = 0.066$ for the latter). Transmission rate of the *t* was similar to the laboratory, for +/*t* × +/*t* crosses (Pearson's $\chi^2 = 2.28$, df = 1, $P = 0.131$), +/*t* males with +/+ females ($\chi^2 = 0.09$, df = 1, $P = 0.761$) and for +/+ males with +/*t* females ($\chi^2 = 0.64$, df = 1, $P = 0.425$).

MHC genotyping

MHC class II loci Aα and Eβ and the MHC class I-linked microsatellite D17Mit28 showed similar levels of variation with 4–5 alleles per locus (summarized in Table 2). At each locus, +/*t* mice had the same allele which was expressed only as a heterozygote variant and was not found in any +/+ mice. Because the *t* haplotype is lethal in its homozygote form, heterozygote excess is anticipated in +/*t* mice. This was confirmed by significant heterozygote excess at the three MHC loci (Fisher's exact tests, $P \leq 0.004$). +/+ mice exhibited a significant heterozygote deficiency at D17Mit 28 (Fisher's exact test, $P = 0.001$) and Eβ (Fisher's exact test, $P = 0.005$), but not at Aα (Fisher's exact test, $P = 0.123$).

Sequences of Aα and Eβ indicate that all alleles were unique not only with respect to nucleotide sequences but

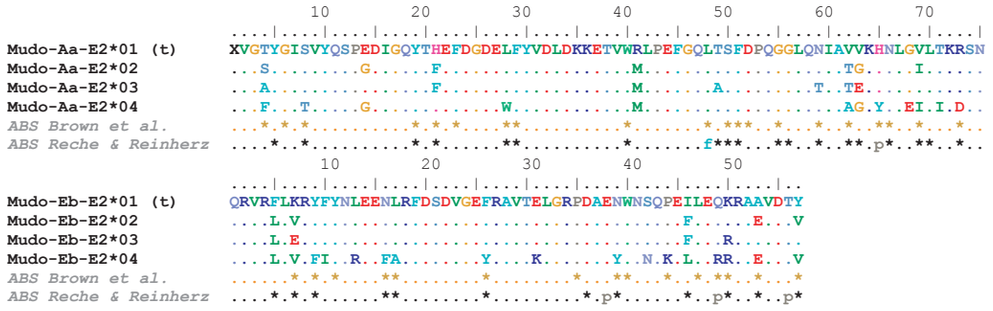

Figure 7. Amino acid sequence of MHC class II genes aligned: (A) Aα and (B) Eβ. Asterisks indicate sites involved in antigen binding identified from Brown et al. (1993) and Reche and Reinherz (2003); p = residues in proximity to the antigen but likely do not contribute to the specificity and binding properties of the molecule, f = residues interacting with flanking regions of the peptide core extending beyond the binding groove (Reche and Reinherz 2003).

Table 3. Variability in three MHC loci and 21 neutral microsatellites for +/*t* and +/+ individuals, and divided into the subset of 12 founders and the subset of 17 randomly chosen individuals.

Type of loci	*N* individuals	*N* loci	Mean *N* alleles/locus	H_{Obs}	H_{Exp}	H_{Obs}–H_{Exp}	*P*
MHC							
+/+	15	3	3.33	0.289	0.526	−0.238	0.001
+/*t*	14	3	4.0	1.000	0.624	0.376	0.000
MHC – excluding founders							
+/+	7	3	3.00	0.286	0.414	−0.128	0.080
+/*t*	10	3	3.33	1.000	0.605	0.394	0.000
Microsatellites							
+/+	15	21	5.29	0.643	0.728	−0.085	0.000
+/*t*	14	21	4.67	0.694	0.615	0.079	0.995
Microsatellites – excluding founders							
+/+	7	21	4.14	0.703	0.720	−0.017	0.955
+/*t*	10	21	4.29	0.705	0.631	0.073	0.999

also at the amino acid level (Fig. 7). Sites involved in antigen binding showed the highest variation with an average of 5.7–7.2 differences in amino acid sequence between alleles compared with 1.8–2.0 differences at neutral sites, indicating that all alleles found might differ in their antigen binding capacities.

These mice were also genotyped for 21 neutral microsatellites. Similar to the pattern observed at the MHC loci, a significant deficit of heterozygotes was found but only for +/+ mice (Table 3, Fisher's exact test, $P < 0.001$) but the difference between observed and expected heterozygosity dropped to a fourth of the value found at MHC loci. To clarify the selective processes acting on the different loci we excluded the 12 founder individuals, and analyzed the remaining random sample of their descendants ($N = 17$). For MHC markers, the excess of heterozygotes in +/*t* remained significant, but a deviation from HW equilibrium was no longer observed in the +/+ population. Neutral markers showed the lowest differences between observed and expected heterozygosity in this sample with no deviation from HW equilibrium, however, a significant heterozygote excess was found in the +/*t* population (Table 3). Differentiation between the +/*t* and +/+ population was significant (Fisher's exact tests, $P < 0.001$) in all analyzed comparisons.

Discussion

Two features of the *t* haplotype are well known: it causes drive in males, and known variants of the *t* haplotype carry a recessive lethal allele. Together, these two phenomena result in a reduction in litter size when +/*t* females produce offspring sired by +/*t* males, a form of genetic incompatibility. The cost and consequences of genetic incompatibility have, however, rarely been measured. Here we have used a combination of laboratory experiments and field data from house mice to do so.

Cost of genetic compatibility

For a +/*t* female or male, mating with a +/*t* rather than a +/+ partner resulted in a litter size reduction of 40%. This

corresponds empirically to a reduction in litter size from seven pups to four pups. Examination of uterine scars showed that the reduction in litter size in $+/t \times +/t$ matings is the result of prenatal mortality, as fertility (the number of implanted embryos) did not differ between crosses. Thus, we found no evidence for reproductive compensation for the loss of homozygous offspring, as has been postulated (Charlesworth 1994). Fecundity, the ability to produce offspring, also did not differ between crosses. Our results are consistent with the presence of a recessive lethal allele within the *t* haplotype, but suggest no additional deleterious effects of the *t* on fertility and fecundity in monogamous matings. This is in contrast with previous studies, which showed reduced fertility in $+/t$ male but not female mice (Lenington et al. 1994; Carroll et al. 2004) and reduced fecundity of $+/t$ females (Carroll et al. 2004). However, selfish genetic elements that affect sperm are generally thought to be associated with a reduction in fertility (Price and Wedell 2008; Wedell 2013).

No mice were found to be homozygous for the *t* haplotype. Although populations are known which host multiple variants of the *t* (Petras 1967; Baker 2008), we found only a single *t* haplotype in our study population.

Drive

Drive of the *t* haplotype amplifies the cost of genetic incompatibility by increasing the frequency at which t/t are produced when both males and females are carriers. In laboratory crosses between $+/t$ and +/+, we found that the *t* haplotype when transmitted solely paternally is inherited by nearly all offspring (a proportion of 0.90), which is consistent with the range of 0.88–0.99 reported in crosses of wild mice (Dunn 1957; Ardlie and Silver 1996; Carroll et al. 2004). In contrast, through females the *t* haplotype was inherited by 0.52 of offspring, consistent with Mendelian expectations and similar to previously reported findings of 0.43–0.45 (Ardlie and Silver 1996; Carroll et al. 2004).

Postcopulatory female choice

The proportion of offspring inheriting the *t* in heterozygous matings was significantly lower, at 0.79, than in matings between $+/t$ males and +/+ females, at 0.90. This reduction in drive has fitness consequences, as more viable offspring will be produced, increasing litter size by 8.3%. Female genetic background thus appears to influence fertilization success of *t* sperm relative to + sperm within the same male. In the only previous study to have compared drive in males according to female *t* genotype within a strain, the proportion of offspring inheriting the *t* in heterozygous matings was 10% lower than in matings between $+/t$ males and +/+ females (Bateman 1960). These two results suggest that in wild mice, the *t* haplotype in females modifies drive in males. Modifiers of drive in natural populations have not been previously found in the *t* haplotype system (Ardlie and Silver 1996), but are known from other selfish genetic elements (Montchamp-Moreau et al. 2001).

Fertilization bias as a result of egg-sperm interactions is a possible mechanism for the lower than expected transmission of the *t* haplotype. Within-male sperm competition is an unlikely mechanism for the fertilization bias, as the bias depends on female genotype. Eggs carrying the *t* will die if fertilized by a *t* sperm, thus all else being equal, selection will favor a mechanism whereby *t* eggs can recognize and avoid fertilization by *t* sperm. Sperm selection is well documented in some sessile hermaphroditic organisms, but there are few examples from other groups (Birkhead and Pizzari 2002, reviewed in Zeh and Zeh 1997). The effect detected in house mice was significant but weak, with an effect size of 10–11% (this study and Bateman 1960).

Precopulatory female choice

Choice of mating partner, rather than choice of sperm, is another way to avoid the cost of fertilizing eggs with genetically incompatible sperm. In free-living house mice, we found that offspring paternity was nonrandom with respect to female and male genotype. $+/t$ males sired 30.0% more offspring with +/+ than with $+/t$ females. Even after accounting for the expected prenatal mortality of t/t pups, the fertilization bias remained (a difference of 17.6%). For +/+ females, higher frequencies of $+/t$ males led to higher rates of paternity by $+/t$ males, but this was not the case for $+/t$ females. It is perhaps surprising that the difference in paternity of $+/t$ and +/+ females is not stronger because of the cost of genetic compatibility to $+/t$ females. Multiple factors can influence paternity outcome: mate choice for a variety of traits at the pre- or postcopulatory stage (e.g., preference for dominant males; Coopersmith and Lenington 1992; Rolland et al. 2003; Carroll et al. 2004), and constraints on females being able to exclusively mate with preferred males, for example because of risk of infanticide by territorial males (Vom Saal and Howard 1982). Environmental variation, such as family genotype, has been previously found to influence preferences of +/+ females, whereas preferences of $+/t$ females for +/+ males persisted (Lenington 1991). Controlled laboratory experiments are needed to clarify our results. Nonetheless, this is the first evidence that $+/t$ females avoid mating with genetically incompatible males in a wild population. Such field evidence is still lacking from other selfish genetic elements.

While we found no differences in postnatal pup mortality in our laboratory study, we cannot reject the possibility of mortality differences in the wild population, which could influence the results of our mate choice analysis and estimates of drive. However, postweaning survival in the wild population is similar between +/*t* and +/+ males, whereas +/*t* females outlive +/+ females (Manser et al. 2011).

Sperm competition

Multiple mating by females could also contribute to a fertilization bias in favor of +/+ males (Haig and Bergstrom 1995), due to the mechanism responsible for drive of the *t* haplotype. Drive is the result of the actions of several loci within the *t* haplotype (Lyon 2003), which act during spermatogenesis when intercellular bridges link syncytial spermatocytes and spermatids (Dym and Fawcett 1971). Products of these loci hyperactivate the sperm mobility kinase allele of + sperm (Herrmann et al. 1999; Bauer et al. 2005, 2007), while *t* sperm are protected by haploid expression of the *t*-specific allele of the same gene (Véron et al. 2009). The disruption of gene regulation in + sperm leads to an impairment of + flagellar function. While this gives an advantage to *t* sperm relative to + sperm in within-ejaculate sperm competition, +/*t* males provide a smaller number of functional sperm relative to +/+ males and are thus at a disadvantage in between-male sperm competition, assuming a fair raffle mechanism (Parker 1998). Lower sperm counts of male mammals have been associated with reduced success in sperm competition (Stockley 1997; Preston et al. 2001). Thus, if +/*t* females did not discriminate between mating partners, but simply mated with multiple males, then there is strong theoretical support that a fertilization bias against +/*t* males would result (Haig and Bergstrom 1995; Manser et al. 2011). Such an effect has been demonstrated in *Drosophila pseudoobscura* that carry a selfish genetic element affecting male sperm (Price et al. 2010), a system in which females are not able to discriminate between carriers and non carriers (Price et al. 2012).

We found two lines of evidence that argue against the sperm competition hypothesis as the sole mechanism for fertilization bias in the context of our paternity analysis. First, our result that +/+ females were more likely to have their offspring sired by +/*t* males when +/*t* males were more abundant in the population, but +/*t* females were not, argues for random mate choice in +/+ females, but not in +/*t* females. Furthermore, when +/*t* males are present at high frequency, then multiple mating will be less effective in avoiding the costs of genetic compatibility. In this context, +/*t* female mate choice for +/+ males is of most benefit. Second, we did not find evidence for a high rate of multiple paternity, however, confidence in our estimates was low due to small sample sizes and small litter sizes. 32% of litters were sired by multiple fathers, and only 15% were sired by both a +/*t* and a +/+ male, which is the context in which sperm competition could produce a fertilization bias. This estimate of multiple paternity is similar to those observed in other studies of wild house mice (12–31% in Dean et al. 2006, 6–43% in Firman and Simmons 2008a), but is relatively low in comparison with other rodent species (Eccard and Wolf 2009). Additional studies are needed to test if multiple mating differs between female genotypes and to estimate the paternity share that results from multiple matings involving males of both genotypes. Moreover, it is important to note that molecular estimates of paternity provide data on male fertilization success, and the actual mating rate (including both successful and unsuccessful males) could be much higher.

MHC – the mechanism underlying fertilization bias?

In summary, we found evidence for a fertilization bias acting in +/*t* females against the *t* at two different stages, one at the level of choice of sire and one at the postcopulatory stage, at the level of choice of sperm. How could such a bias arise? Through comparison of loci within the *t* haplotype, we showed that +/*t* mice carry unique alleles at functionally important genes of the MHC. In contrast, neutral microsatellite markers show no differences in allelic variants between +/*t* and +/+. This is consistent with reports of a unique MHC allele associated with the *t* haplotype in a study of Israeli house mice (Ben-Shlomo et al. 2007), and a study of H2 (MHC) antigens, which detected closely related MHC antigens in different strains of *t* haplotype mice (Figueroa et al. 1985).

The case that the MHC plays a role in fertilization bias is strengthened by evidence that it plays a role in postcopulatory sexual selection. House mouse sperm cells have been reported to express MHC antigens (Fellous and Dausset 1970; Martin-Villa et al. 1999; Ziegler et al. 2002) and olfactory receptor genes (Fukuda et al. 2004). *t* and + sperm and testicular cells have also been found to differ in expressed antigen (Yanagisawa et al. 1974; Cheng et al. 1983), but see (Gable et al. 1979; Goodfellow et al. 1979). Furthermore, female house mice of different genetic backgrounds differ in how quickly they transport sperm (Nicol and McLaren 1974). The *t* haplotype enhances sperm transport after insemination in vivo (Tessler and Olds-Clarke 1981) and influences the rate of egg penetration after insemination in vitro (Olds-Clarke and Carey 1978; Johnson et al. 1995; Redkar et al. 2000). Furthermore, in vitro fertilization experiments have shown nonrandom

fertilization of eggs by sperm of different MHC types (Wedekind et al. 1996; Rülicke et al. 1998), and studies have shown fertilization and pregnancy failures (abortions) when the mating pair shares MHC alleles (Ober et al. 1992; Ho et al. 1994; Apanius et al. 1997; Rülicke et al. 1998). A paternity bias against related sperm in polyandrous kin/non kin matings in house mice is also consistent with avoidance of familial MHC (Firman and Simmons 2008b). Egg-sperm interactions are thought to produce mainly weak fertilization bias, with an effect size of 5% (Rülicke et al. 1998), in line with the 11% fertilization bias we observed in this study between *t* and + sperm from the same male. An alternative hypothesis, the sperm selection hypothesis (Ziegler et al. 2002), proposes an interaction between MHC and chemoreceptors that would result in sperm-egg interactions consistent with MHC-based fertilization bias (Ziegler et al. 2005, 2010).

MHC may also play a role in precopulatory sexual selection. Differences in MHC influence urinary odors in house mice (Carroll et al. 2002; Wilse et al. 2006; Kwak et al. 2009), which are readily detected (Yamazaki et al. 1990; Carroll et al. 2002; Kwak et al. 2009). Olfactory receptors can detect small MHC peptide ligands, in the vomeronasal organ and the main olfactory epithelium (Leinders-Zufall et al. 2004; Boehm and Zufall 2006). Volatile compounds in urine have been related to the MHC (reviewed in Kavaliers et al. 2005 and Kwak et al. 2010) and urine chemistry differs between +/*t* and +/+ males (Drickamer and Lenington 1987; Jemiolo et al. 1991). Female and male house mice have been reported to use odor cues to differentiate between +/*t* and +/+ in Y-maze choice tests (Egid and Lenington 1985; Lenington and Egid 1985). There is evidence for MHC-dependent mate choice in several species including house mice (Roberts 2009; Penn and Musolf 2012), but not every study finds such evidence (Milinski 2006; Penn and Musolf 2012). Where effects are found, they are typically weak (Milinski 2006). Thus, *t*-linked MHC could function as an anti-"green-beard" gene, a marker that individuals who bear it can use to recognize and avoid conspecifics that also bear it, in contrast to a green-beard gene (Dawkins 1976) for an altruistic trait (or kin recognition). However, Lenington and Egid (1985) and Lenington et al. (1988) proposed a compatibility and choice allele system within the *t* haplotype, as they found that females carrying a *t* haplotype recombinant for the distal portion of the fourth inversion, but which retained MHC, no longer showed a mate preference. Recent studies of MHC-linked olfactory receptor genes have shown that they may differ between mouse strains, and coduplicate with MHC loci (Amadou et al. 2003). As the *t* haplotype contains unique MHC alleles, it may also contain unique odor receptor genes. Physical linkage between MHC loci and odor receptor loci on a section of chromosome 17 protected from recombination could allow the evolution of a signal – receiver system as conceived by Lenington and Egid (1985) and Lenington et al. (1988). Olfactory receptor genes are candidates for such a choice allele. Thus, the MHC and its linked polymorphic olfactory receptor genes could provide a potential signal and recognition/mate choice system for the *t* haplotype, both for the individual and for sperm.

An alternative is that the fertilization bias is aimed at the MHC itself, to reduce homozygosity at MHC loci of the offspring. MHC heterozygosity enhances resistance to most infectious agents (reviewed in Penn et al. 2002 and Oliver et al. 2009, but see Ilmonen et al. 2007), increases host survival (Penn et al. 2002), and enhances reproductive success in wild mice (Thoß et al. 2011). MHC homozygosity can be correlated with close inbreeding (Roberts et al. 2006) and inbreeding depression is the most dramatic example of the importance of heterozygosity (Meagher et al. 2000; Keller and Waller 2002). In our sample excluding founder individuals, we found heterozygote excess for +/*t* at MHC and a nonsignificant deficit in +/+, with no difference in neutral microsatellites. A similar excess of heterozygotes at the MHC in +/*t* mice, and a heterozygote deficit in +/+ mice, was detected in an Israeli population, where the *t* haplotype was found at high frequency (Ben-Shlomo et al. 2007).

Individuals carrying the *t* haplotype were always heterozygous for MHC alleles, including the *t*-specific allele which +/+ never carry, and may therefore make attractive mating partners for +/+ individuals. The influence of MHC polymorphism on *t* frequencies has yet to be investigated. Mate choice for partners with different alleles at the MHC would result in a mating pattern whereby fertilizations between +/*t* males and +/*t* females or *t* sperm and *t* eggs are reduced, as they share MHC alleles, but fertilizations between +/*t* and +/+ individuals are not. The higher the frequency of *t* heterozygotes among the breeding population, the stronger should be the effect of discriminatory mate choice in reducing the frequency of transmission of the *t* haplotype to the next generation. Typically, wild mice populations have low *t* frequencies (Ardlie and Silver 1996; Huang et al. 2001), and in such cases one might find no difference in mate choice between +/*t* and +/+ females.

Summary

In this study, we have shown that genetic incompatibility at the *t* haplotype imposes a high cost in terms of embryonic survival. In a population of wild house mice, we have found a weak, but significant, bias in mate choice, which reduces instances of *t*-associated genetic incompatibility. In the laboratory, we have documented a significant

fertilization bias which reduces genetic incompatibility when +/*t* females mate with a +/*t* male. We have shown that a unique MHC allele is associated with the +/*t* haplotype in our study population. Tight linkage between the *t* haplotype and the MHC could be the key to mate choice bias in this system.

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Conflict of Interest

None declared.

References

Amadou, C., R. M. Younger, S. Sims, L. H. Matthews, J. Rogers, A. Kumanovics, et al. 2003. Co-duplication of olfactory receptor and MHC class I genes in the mouse major histocompatibility complex. Hum. Mol. Genet. 12:3025–3040.

Andersson, M., and L. W. Simmons. 2006. Sexual selection and mate choice. Trends Ecol. Evol. 21:296–302.

Apanius, V., D. Penn, P. R. Slev, L. R. Ruff, and W. K. Potts. 1997. The nature of selection on the major histocompatibility complex [Review]. Crit. Rev. Immunol. 17:179–224.

Ardlie, K., and L. M. Silver. 1996. Recent evolution of mouse *t* haplotypes at polymorphic microsatellites associated with the *t* complex responder (*Tcr*) locus. Genet. Res. 67:1–10.

Artzt, K. 1984. Gene mapping within the T/t complex of the mouse III: *t*-lethal genes are arranged in three clusters on chromosome 17. Cell 39:565–572.

Baker, A. E. M. 2008. Mendelian inheritance of *t* haplotypes in house mouse *(Mus musculus domesticus)* field populations. Genet. Res. 90:331–339.

Bateman, N. 1960. Selective fertilization at the T-locus of the mouse. Genet. Res. 1:226–238.

Bates, D., M. Maechler, and B. Bolker. 2011. lme4: Linear mixed-effects models using S4 classes. R package version 0.999375-39. Available at http://CRAN.R-project.org/package=lme4 (accessed December 3, 2011).

Bauer, H., J. Willert, B. Koschorz, and B. G. Herrmann. 2005. The *t*-complex-encoded GTPase-activating protein Tagap1 acts as a transmission ratio distorter in mice. Nat. Genet. 37:969–973.

Bauer, H., N. Véron, J. Willert, and B. G. Herrmann. 2007. The *t*-complex-encoded guanine nucleotide exchange factor *Fgd2* reveals that two opposing signaling pathways promote transmission ratio distortion in the mouse. Genes Dev. 21:143–147.

Ben-Shlomo, R., E. Neufeld, D. Berger, S. Lenington, and U. Ritte. 2007. The dynamic of the *t*-haplotype in wild populations of the house mouse *Mus musculus domesticus* in Israel. Mamm. Genome 18:164–172.

Berry, R. J., F. H. Tattersall, and J. Hurst. 2008. Genus *Mus*. Pp. 141–149 *in* S. Harris and D. W. Yalden, eds. Mammals of the British Isles Handbook. 4th ed. The Mammal Society, Southampton, U.K.

Birkhead, T. R., and T. Pizzari. 2002. Postcopulatory sexual selection. Nat. Rev. Genet. 3:262–273.

Boehm, T., and F. Zufall. 2006. MHC peptides and the sensory evaluation of genotype. Trends Neurosci. 29:100–107.

Brown, J. H., T. S. Jardetzky, J. C. Gorga, L. J. Stern, R. G. Urban, J. L. Strominger, et al. 1993. Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. Nature 364:33–39.

Bult, C. J., J. T. Eppig, J. A. Kadin, J. E. Richardson, and J. A. Blake. 2008. Group atmotMGD. The Mouse Genome Database (MGD): mouse biology and model systems. Nucleic Acids Res. 36:D724–D728.

Carroll, L. S., D. J. Penn, and W. K. Potts. 2002. Discrimination of MHC-derived odors by untrained mice is consistent with divergence in peptide-binding region residues. Proc. Natl. Acad. Sci. USA 99:2187–2192.

Carroll, L. S., S. Meagher, L. Morrison, D. J. Penn, and W. K. Potts. 2004. Fitness effects of a selfish gene (the *Mus t* complex) are revealed in an ecological context. Evolution 58:1318–1328.

Charlesworth, B. 1994. The evolution of lethals in the *t*-haplotype system of the mouse. Proc. Biol. Sci. 258:101–107.

Cheetham, S. A., M. D. Thom, F. Jury, W. E. R. Ollier, R. J. Beynon, and J. L. Hurst. 2007. The genetic basis of individual-recognition signals in the mouse. Curr. Biol. 17:1771–1777.

Cheng, C. C., K. Sege, A. K. Alton, D. Bennett, and K. Artzt. 1983. Characterization of an antigen present on testicular cells and pre-implantation embryos whose expression is modified by the t12 haplotype. Int. J. Immunogenet. 10:465–485.

Chesley, P., and L. C. Dunn. 1936. The inheritance of taillessness (anury) in the house mouse. Genetics 21:525–536.

Coopersmith, C. B., and S. Lenington. 1992. Female preferences based on male quality in house mice – interaction between male-dominance rank and T-Complex genotype. Ethology 90:1–16.

Dawkins, R. 1976. The selfish gene. Oxford Univ. Press, Oxford, U.K.

Dean, M. D., K. G. Ardlie, and M. W. Nachman. 2006. The frequency of multiple paternity suggests that sperm

competition is common in house mice *(Mus domesticus)*. Mol. Ecol. 15:4141–4151.

Drickamer, L. C., and S. Lenington. 1987. T-locus effects on the male urinary chemosignal that accelerates puberty in female mice. Anim. Behav. 35:1581–1583.

Dunn, L. C. 1937. A third lethal in the T (Brachy) series in the house mouse. Proc. Natl. Acad. Sci. USA 23:474–477.

Dunn, L. C. 1957. Evidence of evolutionary forces leading to the spread of lethal genes in wild populations house mice. Proc. Natl. Acad. Sci. USA 43:158–163.

Dym, M., and D. W. Fawcett. 1971. Further observations on the numbers of spermatogonia, spermatocytes, and spermatids connected by intercellular bridges in the mammalian testis. Biol. Reprod. 4:195–215.

Eccard, J. A., and J. B. W. Wolf. 2009. Effects of brood size on multiple-paternity rates: a case for 'paternity share' as an offspring-based estimate. Anim. Behav. 78:563–571.

Egid, K., and S. Lenington. 1985. Responses of male mice to odors of females: effects of T- and H-2-locus genotype. Behav. Genet. 15:287–295.

Fellous, M., and J. Dausset. 1970. Probable haploid expression of *HL-A* antigens on human spermatozoon. Nature 225:191–193.

Figueroa, F., M. Golubić, D. Nižetić, and J. Klein. 1985. Evolution of mouse major histocompatibility complex genes borne by *t* chromosomes. Proc. Natl. Acad. Sci. USA 82:2819–2823.

Firman, R. C., and L. W. Simmons. 2008a. The frequency of multiple paternity predicts variation in testes size among island populations of house mice. J. Evol. Biol. 21:1524–1533.

Firman, R. C., and L. W. Simmons. 2008b. Polyandry facilitates postcopulatory inbreeding avoidance in house mice. Evolution 62:603–611.

Fox, J. 1997. Applied regression analysis, linear models and related methods. Sage, Thousand Oaks, CA.

Fukuda, N., K. Yomogida, M. Okabe, and K. Touhara. 2004. Functional characterization of a mouse testicular olfactory receptor and its role in chemosensing and in regulation of sperm motility. J. Cell Sci. 117:5835–5845.

Gable, R. J., J. R. Levinson, H. O. McDevitt, and P. N. Goodfellow. 1979. Assay for antibody mediated cytotoxicity of mouse spermatozoa by Rb-86 release. Tissue Antigens 13:177–185.

Goodfellow, P. N., J. R. Levinson, R. J. Gable, and H. O. McDevitt. 1979. Analysis of anti-sperm sera for *T/t* locus-specific antibody. J. Reprod. Immunol. 1:11–21.

Haig, D., and C. T. Bergstrom. 1995. Multiple mating, sperm competition and meiotic drive. J. Evol. Biol. 8:265–282.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. 41:95–98.

Hammer, M. F., J. Schimenti, and L. M. Silver. 1989. Evolution of mouse chromosome 17 and the origin of inversions associated with *t* haplotypes. Proc. Natl. Acad. Sci. USA 86:3261–3265.

Herrmann, B. G., B. Koschorz, K. Wertz, J. McLaughlin, and A. Kispert. 1999. A protein kinase encoded by the *t complex responder* gene causes non-mendelian inheritance. Nature 402:141–146.

Ho, H. N., Y. S. Yang, R. P. Hsieh, H. R. Lin, S. U. Chen, H. F. Chen, et al. 1994. Sharing of human-leukocyte antigens in couples with unexplained infertility affects the success of in-vitro fertilization and tubal embryo-transfer. Am. J. Obstet. Gynecol. 170:63–71.

Huang, S.-W., K. G. Ardlie, and H.-T. Yu. 2001. Frequency and distribution of *t*-haplotypes in the Southeast Asian house mouse (*Mus musculus castaneus*) in Taiwan. Mol. Ecol. 10:2349–2354.

Ilmonen, P., D. J. Penn, K. Damjanovich, L. Morrison, L. Ghotbi, and W. K. Potts. 2007. Major histocompatibility complex heterozygosity reduces fitness in experimentally infected mice. Genetics 176:2501–2508.

Jemiolo, B., T.-M. Xie, F. Andreolini, A. E. M. Baker, and M. Novotny. 1991. The *t* complex of the mouse: chemical characterization by urinary volatile profiles. J. Chem. Ecol. 17:353–367.

Johnson, L. R., S. H. Pilder, J. L. Bailey, and P. Olds-Clarke. 1995. Sperm from mice carrying one or two *t* haplotypes are deficient in investment and oocyte penetration. Dev. Biol. 168:138–149.

Jordan, W. C., and M. W. Bruford. 1998. New perspectives on mate choice and the MHC. Heredity 81:127–133.

Kalinowski, S. T., M. L. Taper, and T. C. Marshall. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol. Ecol. 16:1099–1106.

Kavaliers, M., E. Choleris, and D. W. Pfaff. 2005. Genes, odours and the recognition of parasitized individuals by rodents. Trends Ecol. Evol. 21:423–429.

Keller, L. F., and D. M. Waller. 2002. Inbreeding effects in wild populations. Trends Ecol. Evol. 17:230–241.

Klein, J., P. Sipos, F. Figureoa. 1984. Polymorphism of t-complex genes in European wild mice. Genet. Res. 44:39–46.

König, B., and A. K. Lindholm. 2012. The complex social environment of female house mice (*Mus domesticus*). Pp. 114–134 *in* M. Malochán, S. J. E. Baird, P. Munclinger, and J. Piálek, eds. Evolution of the house mouse. Cambridge Univ. Press, Cambridge, U.K.

Krackow, S. 1990. Sex-specific embryonic mortality during concurrent pregnancy and lactation in house mice. J. Exp. Zool. 256:106–112.

Krackow, S. 1992. Sex ratio manipulation in wild house mice: the effect of fetal resorption in relation to the mode of reproduction. Biol. Reprod. 47:541–548.

Kwak, J., M. C. Opiekun, K. Matsumura, G. Preti, K. Yamazaki, and G. K. Beauchamp. 2009. Major histocompatibility complex-regulated odortypes: peptide-free urinary volatile signals. Physiol. Behav. 96:184–188.

Kwak, J., A. Willse, G. Preti, K. Yamazaki, and G. K. Beauchamp. 2010. In search of the chemical basis for MHC odourtypes. Proc. Biol. Sci. 277:2417–2425.

Leinders-Zufall, T., P. Brennan, P. Widmayer, S. P. Chandramani, A. Maul-Pavicic, M. Jäger, et al. 2004. MHC class I peptides as chemosensory signals in the vomeronasal organ. Science 306:1033–1037.

Lenington, S. 1991. The *t* complex: a story of genes, behavior, and populations. Adv. Study Behav. 20:51–86.

Lenington, S., and C. Coopersmith. 1992. Genetic basis of mating preferences in wild house mice. Am. Zool. 32:40–47.

Lenington, S., and K. Egid. 1985. Female discrimination of male odors correlated with male genotype at the *T* locus: a response to *T*-locus or *H-2*-locus variability? Behav. Genet. 15:53–67.

Lenington, S., K. Egid, and J. Williams. 1988. Analysis of a genetic recognition system in wild house mice. Behav. Genet. 18:549–564.

Lenington, S., C. B. Coopersmith, and M. Erhart. 1994. Female preference and variability among *t*-haplotypes in wild house mice. Am. Nat. 143:766–784.

Lyon, M. F. 2003. Transmission ratio distortion in mice. Annu. Rev. Genet. 37:393–408.

Manser, A., A. K. Lindholm, B. König, and H. Bagheri. 2011. Polyandry and the decrease of a selfish genetic element in a wild house mouse population. Evolution 65:2435–2447.

Martin-Villa, J. M., J. Longás, and A. Arnáiz-Villena. 1999. Cyclic expression of HLA class I and II molecules on the surface of purified human spermatozoa and their control by serum inhibin B levels. Biol. Reprod. 61:1381–1386.

Mays, H. L., Jr., and G. E. Hill. 2004. Choosing mates: good genes versus genes that are a good fit. Trends Ecol. Evol. 19:554–559.

Meagher, S., and W. K. Potts. 1997. A microsatellite-based MHC genotyping system for house mice (*Mus domesticus)*. Hereditas. 127:75–82.

Meagher, S., D. J. Penn, and W. K. Potts. 2000. Male-male competition magnifies inbreeding depression in wild house mice. Proc. Natl. Acad. Sci. USA 97:3324–3329.

Milinski, M. 2006. The major histocompatibility complex, sexual selection, and mate choice. Annu. Rev. Ecol. Evol. Syst. 37:159–186.

Montchamp-Moreau, C., V. Ginhoux, and A. Atlan. 2001. The Y chromosomes of *Drosophila simulans* are highly polymorphic for their ability to suppress sex-ratio drive. Evolution 55:728–737.

Müllenbach, R., P. J. Lagoda, C. Welter.1989. An efficient salt-chloroform extraction of DNA from blood and tissues. Trends Genet. 5:391.

Neff, B. D., and T. E. Pitcher. 2005. Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. Mol. Ecol. 14:19–38.

Nicol, A., and A. McLaren. 1974. An effect of the female genotype on sperm transport in mice. J. Reprod. Fertil. 39:421–424.

Ober, C., S. Elias, D. D. Kostyu, and W. W. Hauck. 1992. Decreased fecundability in Hutterite couples sharing HLA-DR. Am. J. Hum. Genet. 50:6–14.

Olds-Clarke, P., and J. E. Carey. 1978. Rate of egg penetration in vitro accelerated by *T/t* locus in the mouse. J. Exp. Zool. 206:323–331.

Oliver, M. K., S. Telfer, and S. B. Piertney. 2009. Major histocompatibility complex (MHC) heterozygote superiority to natural multi-parasite infections in the water vole (*Arvicola terrestris*). Proc. Biol. Sci. 276:1119–1128.

Parker, G. A. 1998. Sperm competition and the evolution of ejaculates: towards a theory base. Pp. 3–53 *in* T. R. Birkhead and A. P. Møller, eds. Sperm competition and sexual selection. Academic Press, San Diego, CA.

Penn, D. J. 2002. The scent of genetic compatibility: sexual selection and the major histocompatibility complex. Ethology 108:1–21.

Penn, D. J., and K. Musolf. 2012. The evolution of MHC diversity in house mice. Pp. 221–252 *in* M. Macholán, S. J. E. Baird, P. Munclinger, and J. Piálek, eds. Evolution of the house mouse. Cambridge Univ. Press, Cambridge, U.K.

Penn, D. J., K. Damjanovich, and W. K. Potts. 2002. MHC heterozygosity confers a selective advantage against multiple-strain infections. Proc. Natl. Acad. Sci. USA 99:11260–11264.

Petras, M. L. 1967. Studies of natural populations of *Mus*. II. Polymorphism at the T locus. Evolution 21:466–478.

Preston, B. T., I. R. Stevenson, J. M. Pemberton, and K. Wilson. 2001. Dominant rams lose out by sperm depletion – a waning success in siring counters a ram's high score in competition for ewes. Nature 409:681–682.

Price, T. A. R., and N. Wedell. 2008. Selfish genetic elements and sexual selection: their impact on male fertility. Genetica 134:99–111.

Price, T. A. R., G. D. D. Hurst, and N. Wedell. 2010. Polyandry prevents extinction. Curr. Biol. 20:1–5.

Price, T. A. R., Z. Lewis, D. T. Smith, G. D. D. Hurst, and N. Wedell. 2012. No evidence of mate discrimination against males carrying a sex ratio distorter in *Drosophila pseudoobscura*. Behav. Ecol. Sociobiol. 66:561–568.

Puurtinen, M., T. Ketola, and J. S. Kotiaho. 2005. Genetic compatibility and sexual selection. Trends Ecol. Evol. 20:157–158.

Puurtinen, M., T. Ketola, and J. S. Kotiaho. 2009. The good-genes and compatible-genes benefits of mate choice. Am. Nat. 174:741–752.

R Development Core Team. 2011. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J. Hered. 86:248–249.

Reche, P. A., and E. L. Reinherz. 2003. Sequence variability analysis of human class I and class II MHC molecules: functional and structural correlates of amino acid polymorphisms. J. Mol. Biol. 331:623–641.

Redkar, A. A., Y. Si, S. N. Twine, S. H. Pilder, and P. Olds-Clarke. 2000. Genes in the first and fourth inversions of the mouse *t* complex synergistically mediate sperm capacitation and interactions with the oocyte. Dev. Biol. 226:267–280.

Roberts, S. C. 2009. Complexity and context of MHC-correlated mating preferences in wild populations. Mol. Ecol. 18:3121–3123.

Roberts, S. C., M. L. Hale, and M. Petrie. 2006. Correlations between heterozygosity and measures of genetic similarity: implications for understanding mate choice. J. Evol. Biol. 19:558–569.

Rolland, C., D. W. Macdonald, and M. de Fraipont. 2003. Free female choice in house mice: leaving best for last. Behaviour 140:1371–1388.

Rousset, F. 2008. genepop '007: a complete reimplementation of the Genepop software for Windows and Linus. Mol. Ecol. Resour. 8:103–106.

Rülicke, T., M. Chapuisat, F. R. Homberger, E. Macas, and C. Wedekind. 1998. MHC-genotype of progeny influenced by parental infection. Proc. Biol. Sci. 265:711–716.

Schad, J., S. Sommer, and J. U. Ganzhorn. 2004. MHC variability of a small lemur in the littoral forest fragments of southeastern Madagascar. Conserv. Genet. 5:299–309.

Schimenti, J., and M. Hammer. 1990. Rapid identification of mouse *t* haplotypes by PCR polymorphism (PCRP). Mouse Genome 87:108.

Sherborne, A. L., M. D. Thom, S. Paterson, F. Jury, W. E. R. Ollier, P. Stockley, et al. 2007. The genetic basis of inbreeding avoidance in house mice. Curr. Biol. 17:2061–2066.

Silver, L. M. 1993. The peculiar journey of a selfish chromosome: mouse *t* haplotypes and meiotic drive. Trends Genet. 9:250–254.

Stearns, S. C. 1987. The selection-arena hypothesis. Pp. 337–349 *in* S. C. Stearns, ed. The evolution of sex and its consequences. Birkhäuser Verlag, Basel.

Stockley, P. 1997. No evidence of sperm selection by female common shrews. Proc. Biol. Sci. 264:1497–1500.

Teschke, M., O. Mukabayire, T. Wiehe, and D. Tautz. 2008. Identification of selective sweeps in closely related populations of the house mouse based on microsatellite scans. Genetics 180:1537–1545.

Tessler, S., and P. Olds-Clarke. 1981. Male genotype influences sperm transport in female mice. Biol. Reprod. 24:806–813.

Theiler, K. 1989. The house mouse atlas of embryonic development. Springer-Verlag, New York.

Thoß, M., P. Ilmonen, K. Musolf, and D. J. Penn. 2011. Major histocompatibility complex heterozygosity enhances reproductive success. Mol. Ecol. 20:1546–1557.

Tregenza, T., and N. Wedell. 2000. Genetic compatibility, mate choice and patterns of parentage: invited review. Mol. Ecol. 9:1013–1027.

Venables, W. N., and B. D. Ripley. 2002. Modern applied statistics with S. Springer, New York.

Véron, N., H. Bauer, A. Y. Weiße, G. Lüder, M. Werber, and B. G. Herrmann. 2009. Retention of gene products in syncytial spermatids promotes non-Mendelian inheritance as revealed by the *t* complex responder. Genes Dev. 23:2705–2710.

Vom Saal, F. S., and L. S. Howard. 1982. The regulation of infanticide and parental behaviour: implications for reproductive success in male mice. Science 215:1270–1272.

Wedekind, C., M. Chapuisat, E. Macas, and T. Rulicke. 1996. Non-random fertilization in mice correlates with the MHC and something else. Heredity 77:400–409.

Wedell, N. 2013. The dynamic relationship between polyandry and selfish genetic elements. Philos. Trans. R. Soc. Lond. B Biol. Sci. 368:20120049.

Williams, J. R., and S. Lenington. 1993. Factors modulating preferences of female house mice for males differing in *t*-complex genotype: role of *t*-complex genotype, genetic background, and estrous condition of females. Behav. Genet. 23:51–58.

Wilse, A., J. Kwak, K. Yamazaki, G. Preti, J. H. Wahl, and G. K. Beauchamp. 2006. Individual odortypes: interaction of MHC and background genes. Immunogenetics 58:967–982.

Yamazaki, K., and G. K. Beauchamp. 2007. Genetic basis for MHC-dependent mate choice. Adv. Genet. 59:129–145.

Yamazaki, K., M. Yamaguchi, L. Baranoski, J. Bard, E. A. Boyse, and L. Thomas. 1979. Recognition among mice: evidence from the use of a Y-maze differentially scented by congenic mice of different major histocompatibility types. J. Exp. Med. 150:755–760.

Yamazaki, K., G. K. Beauchamp, J. Bard, and E. A. Boyse. 1990. Single MHC gene mutations alter urine odour constitution in mice. Pp. 255–259 *in* D. W. Macdonald, D. Müller-Schwarze, and S. E. N, eds. Chemical signals in vertebrates. Oxford Univ. Press, Oxford, U.K.

Yanagisawa, K., D. Bennett, E. A. Boyse, L. C. Dunn, and A. Dimeo. 1974. Serological identification of sperm antigens specified by lethal *t*-alleles in the mouse. Immunogenetics 1:57–67.

Zeh, J. A., and D. W. Zeh. 1997. The evolution of polyandry II: post-copulatory defences against genetic compatibility. Proc. Biol. Sci. 264:69–75.

Ziegler, A., G. Dohr, and B. Uchanska-Ziegler. 2002. Possible roles for products of polymorphic MHC and linked olfactory receptor genes during selection processes in reproduction. Am. J. Reprod. Immunol. 48:34–42.

Ziegler, A., H. Kentenich, and B. Uchanska-Ziegler. 2005. Female choice and the MHC. Trends Immunol. 26:496–502.

Ziegler, A., P. S. C. Santos, T. Kellermann, and B. Uchanska-Ziegler. 2010. Self/Nonself perception, reproduction and the extended MHC. Landes Biosci. 1:176–191.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Litter sizes and placental scar counts at birth resulting from different mating crosses.

Supplementary Table 1. Litter sizes and placental scar counts at birth resulting from different mating crosses. Total scars indicate the total number of implanted embryos, red scars reflect the numbers of these embryos surviving to birth, and yellow scars indicate the number of these embryos which died prenatally.

Mating cross (female X male)	N	Mean ± SE of birth litter size	Mean ± SE of total scars	Mean ± SE of red scars	Mean ± SE of yellow scars
Exp. 1					
+/*t* X +/*t*	11	3.45 ± 0.46			
+/*t* X +/+	12	5.33 ± 0.83			
+/+ X +/*t*	14	5.86 ± 0.50			
+/+ X +/+	16	5.75 ± 0.30			
Exp. 2					
+/*t* X +/*t*	22	3.95 ± 0.39	7.27 ± 0.26	4.18 ± 0.42	3.09 ± 0.37
+/*t* X +/+	21	7.00 ± 0.34	7.67 ± 0.28	7.00 ± 0.35	0.67 ± 0.29
+/+ X +/*t*	19	6.21 ± 0.46	7.47 ± 0.32	6.63 ± 0.38	0.84 ± 0.28
+/+ X +/+	12	7.25 ± 0.37	8.17 ± 0.30	7.75 ± 0.31	0.42 ± 0.15
Combined					
+/*t* X +/*t*	33	3.78 ± 0.30			
+/*t* X +/+	33	6.39 ± 0.39			
+/+ X +/*t*	33	6.06 ± 0.34			
+/+ X +/+	28	6.39 ± 0.27			

Chapter 11

Polyandry and the decrease of a selfish genetic element in a wild house mouse population

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ORIGINAL ARTICLE

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POLYANDRY AND THE DECREASE OF A SELFISH GENETIC ELEMENT IN A WILD HOUSE MOUSE POPULATION

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Despite deleterious effects on individuals, the *t* haplotype is a selfish genetic element present in many house mouse populations. By distorting the transmission ratio, +/*t* males transmit the *t* haplotype to up to 90% of their offspring. However, *t*/*t* individuals perish in utero. Theoretical models based on these properties predict a much higher *t* frequency than observed, leading to the *t* paradox. Here, we use empirical field data and theoretical approaches to investigate whether polyandry is a female counterstrategy against the negative fitness consequences of such distorters. We found a significant decrease of the *t* frequency over a period of 5.5 years that cannot be explained by the effect of transmission ratio distortion and recessive lethals, despite significantly higher life expectancy of +/*t* females compared to +/+ females. We quantified life-history data and homozygous and heterozygous fitness effects. Population subdivision and inbreeding were excluded as evolutionary forces influencing the *t* system. The possible influence of polyandry on the *t* system was then investigated by applying a stochastic model to this situation. Simulations show that polyandry can explain the observed *t* dynamics, making it a biologically plausible explanation for low *t* frequencies in natural populations in general.

KEY WORDS: generation time, intragenomic conflict, *t* haplotype, *t* frequency paradox, overdominance.

In its classical conception, Darwinian evolution by natural selection predicts that genes have to contribute to organismal fitness to be successful. An increasing number of cases are emerging where this paradigm is violated. Selfish genetic elements define such heritable entities. They spread through populations despite being associated with negative fitness consequences for the organism (Burt and Trivers 2006). These stretches of DNA distort Mendelian segregation in their favor (transmission ratio distortion (TRD) or meiotic drive) and thereby gain an advantage over their wild-type variants. Selfish genetic elements fascinate evolutionary biologists because they demonstrate persuasively, that systematic advantages at a gene or gamete level can lead to a distinct disadvantage at a higher level of organization such as the individual or population (Okasha 2006).

The t haplotype. Since its discovery in 1927 (Dobrovolskaia-Zavadskaia and Kobozieff 1927), the *t* haplotype in house mice *(Mus domesticus)* has been intensively studied and is now the best known example of transmission ratio distorters. Efforts to understand the underlying genetics of this distortion have been quite successful. Heterozygote males produce equal

proportions of + and *t* sperm (Silver and Olds-Clarke 1984), but an elaborate poison antidote system exclusively impairs flagellar function of the + sperm (Schimenti 2000; Lyon 2003; Bauer et al. 2005, 2007). This leads to a transmission of *t* gametes that deviates considerably from 50%, and up to as much as 99% (Silver 1993; Lyon 2003). Females, on the other hand, transmit the *t* gametes in the usual Mendelian ratios. The *t* haplotype comprises a complex of linked genes as large as 20 cM (30–40 Mbp), occupying the proximal third of chromosome 17 (Silver 1993). Four major, nonoverlapping inversions block recombination and assure that the complex is transmitted as one intact entity (Artzt et al. 1982). It has been found in all four house mouse subspecies across the world and is thought to have existed in house mouse populations for 1.5 to 2 million years (Silver 1993). As is the case for most known distorter systems, they have negative fitness effects on an individual level. Cases with positive or neutral effects are probably not detected because they will drive to fixation in very short time. Numerous variants of *t* haplotypes have been found. Most t haplotypes carry lethal recessive mutations such that homozygotes for the same variant perish in utero. On the other hand, homozygosity for different, complementing variants (e.g., t^x/t^y) invariably result in male sterility (Klein et al. 1984).

The t frequency paradox. Despite the solid understanding of the structure and mechanisms, the implications for population dynamics of this genetic polymorphism are still a puzzle (see Ardlie (1998) for a review). In one of the first theoretical approaches to this system more than 50 years ago, Bruck (1957) provided a mathematical model that took into account the antagonism between segregation distortion in males supporting the *t* haplotype frequency, and viability selection at the embryonic stage acting against it. The model assumes random mating and an infinite, unstructured population. Under these assumptions, the frequency of the *t* haplotype reaches a steady-state equilibrium that is surprisingly high, despite the strong negative fitness consequences for t/t individuals. With a TRD of 0.9, an equilibrium *t* frequency of 0.33 is predicted, meaning that two-thirds of a population are expected to be $+/t$ heterozygotes. This expectation is however in marked contrast to frequencies usually found in wild populations. Various empirical studies measuring the frequency of *t* haplotypes in natural populations of different subspecies around the globe show the same picture: frequencies are on a persistent but low level, with *t* frequencies ranging between 0.05 and 0.15 (Ardlie and Silver 1998; Lenington et al. 1988; Huang et al. 2001; Dod et al. 2003). This discrepancy—often called the *t* frequency paradox—has evoked a large number of theoretical models (both analytical models and stochastic simulations) focusing on various forces that might account for this low frequency in a natural context.

Population subdivision. Population subdivision and genetic drift were considered as possible factors to reduce *t* haplotype frequency, as they reduce heterozygosity and thereby the frequency of *t* carrying individuals (Lewontin and Dunn 1960; Levin et al. 1969; Petras and Topping 1983; Nunney 1993). It has nevertheless been a matter of debate whether mouse populations are as substructured as assumed by these models. Some studies point out that overall *t* frequencies within a population are rather stable (Lenington et al. 1988), whereas empirical data indicate that population size has an influence on *t* allele frequency, with frequencies decreasing in larger populations (Ardlie and Silver 1998).

Heterozygote fitness effects. Selection against $+/t$ heterozygotes will substantially reduce *t* frequencies (Young 1967; Lewontin 1968; Johnston and Brown 1969; Hartl 1970). Here again, empirical data do not show a clear picture. Besides the expected reduction in litter size after a double $+/t$ mating, several studies also showed litter size reductions if only one sex was carrying a *t* (Johnston and Brown 1969; Lenington et al. 1994; Carroll et al. 2004). Viability, on the other hand, has been found to be either higher (Dunn et al. 1958) or lower (Carroll et al. 2004) in $+/t$ heterozygotes independent of sex.

Female choice. In a series of studies, Lenington and collaborators found evidence for sexual selection against *t* carrying males via female preferences (Lenington and Egid 1985; Lenington et al. 1994). Using a Y-maze apparatus, they showed repeatedly that $+/t$ females spend significantly more time near $+/+$ male-derived odor cues when given the choice between $+/+$ and $+/t$. This preference is adaptive in light of the reduction in litter size that would result from a mating with a $+/t$ male. However, the question whether these odor preferences reflect actual mating preference is unresolved. Furthermore, male dominance status seems to be an even more important predictor for female olfactory preference (Coopersmith and Lenington 1992), although evidence of the effect of *t* on male dominance is contradictory (Lenington et al. 1996; Carroll et al. 2004).

Genetic modifiers. Theoretical work predicts the evolution of genetic modifiers suppressing segregation distortion (Charlesworth and Hartl 1978). Such modifiers have been found in various other distorter systems (Hiraizumi and Thomas 1984; Atlan et al. 2003). Surprisingly, they appear to be rare for *t* haplotypes of natural house mouse populations (Ardlie and Silver 1996), although there are reports on suppressors from laboratory mice (Bennett et al. 1983; Gummere et al. 1986).

Polyandry. Even though a reduction of the segregation ratio due to modifiers seems to be nearly absent in wild populations, there still remains the possibility of a reduction of TRD by other means, such as polyandry. Haig and Bergstrom (1995) proposed the idea that females could avoid individual fitness reduction due to distortion by systematic multiple mating. The hypothesis of polyandry as a general counterstrategy against distorters is based on the observation that gametes carrying the *t* haplotype are

"by definition" strong intraejaculate competitors, but do comparatively poorly in competition with other ejaculates (Zeh and Zeh 1996; Zeh and Zeh 1997; Price and Wedell 2008). In comparison to previously described precopulatory mating preferences, this mechanism would not even require the female ability to distinguish between the two different genotypes. Hence, polyandry is a simpler and potentially more robust strategy to counter the spread of *t* haplotypes. A series of empirical studies on several species, predominantly on the genus *Drosophila*, support this idea. Both fertility reductions and reduced competitive ability of sperm from males carrying selfish genetic elements have been found in various systems (see Price and Wedell (2008) for an overview). In *Drosophila pseudoobscura* the X-linked driver (SR) was found to reduce sperm competitive ability (Price et al. 2008a) and females evolved increased remating rates in the presence of the distorter (Price et al. 2008b). Fertility reduction and negative effects on sperm competitive abilities were also found in the SR system and *Wolbachia* of *Drosophila simulans* (Snook et al. 2000; Atlan et al. 2004; Champion de Crespigny and Wedell 2006).

This hypothesis appears to be quite promising for the house mouse case, because recent studies indicate that female house mice are indeed actively polyandrous both in the wild (Dean et al. 2006) and in an experimental context (Rolland et al. 2003). Polyandry provides opportunity for postcopulatory selection processes such as sperm competition and cryptic female choice. It has already been shown that polyandry increases offspring postbirth survival (Firman and Simmons 2008c) and facilitates inbreeding avoidance (Firman and Simmons 2008b). As mentioned above, evidence for negative effects of the *t* on male fertility are convincing: apart from t/t sterility (in the case of complementing *t* variants), reduced fertility (of about 20%) has invariably been reported in $+/t$ heterozygous males (Johnston and Brown 1969; Lenington et al. 1994; Carroll et al. 2004). However, empirical data on sperm competitive abilities in relation to the *t* haplotype are still scarce. One study looking at paternities in a wild population yielded a mean fraction of 0.17 $+/t$ among litters involving both $+/t$ and $+/+$ fathers (based on three litters, Ardlie and Silver (1996)), another study using controlled sperm mixing experiments obtained a $+/t$ proportion of 0.22 (based on eight litters, Olds-Clarke and Peitz (1986)). In the study of Carroll et al. (2004) on seminatural enclosure populations, the *t* was transmitted to 36% of the offspring.

Aims. The present study focuses on both empirical and theoretical approaches to understanding the dynamics of the *t* haplotype on a specific wild house mouse population. First, we provide a theoretical model looking at possible impacts of polyandry on *t* haplotype frequency. To our knowledge, no theoretical model has investigated this for *t* haplotypes. However, the evidence in the previous section make polyandry a promising evolutionary force to explain low *t* frequencies in natural populations. Second, extensive data collection in a free-living population of house mice over a time period of more than five years allows us to estimate many parameters likely to affect the *t* frequency. In addition to well-described parameters such as distortion level and homozygous fitness effects, we were able to obtain reliable estimates on heterozygous fitness effects, the degree of inbreeding, as well as *t* frequency dynamics. Reliable estimates of these have not been available for natural house mouse populations so far (Burt and Trivers 2006). In addition, we were able to describe a series of general life-history parameters (generation time, life expectancy, net reproductive rate) as yet largely unknown for wild house mice. In a third step, the theoretical model and the empirical data were compared. We ran computer simulations using the parameters estimated from our population to predict the *t* frequency dynamics. With the present approach, we are able to test specific models with parameters directly estimated from the population of interest.

Materials and Methods

THE MODEL

A classical Fisher–Wright population with infinite population size and no mutation is assumed. For the purposes of the model, the whole *t* haplotype is simplified to a single locus with two alleles—the wild-type allele $+$ and the distorter allele *t*. Using $i = 1$ for females and $i = 2$ for males, the variables $p_{+(i)}$ and $p_{t(i)}$ for each sex denote the frequency of alleles $+$ and *t*, respectively. Similarly, for each sex *i*, the variables $P_{++(i)}$, $P_{+t(i)}$, and $P_{tt(i)}$ describe the frequency of the genotypes $+/+$, $+/t$, and t/t, respectively. For example $P_{++(1)}$ would represent the genotype frequency of female homozygote wild-types. t/t homozygotes are inviable and it simply holds that $p_{t(i)} = \frac{1}{2} P_{+t(i)}$ and $P_{++(i)} = 1 - P_{+t(i)}$. This also explains why $p_{t(i)}$ can never exceed 0.5, the case in which all members of the population are heterozygotes. The sex-independent *t* allele frequency p_t can be expressed as $p_t = f_1 p_{t(1)} + f_2 p_{t(2)}$, where f_1 and f_2 are relative frequencies of females and males in the population ($f_1 + f_2 = 1$).

The Life Cycle. To determine the allele frequencies $p'_{t(i)}$ in the next, nonoverlapping generation, the following life cycle was used.

(1) First, adult individuals of the present generation mate with each other. Mating probabilities are based on the genotype frequencies, which means that all matings are random. A certain fraction of the females $0 \leqslant \psi \leqslant 1$ is assumed to mate twice, whereas the rest of the female population $1 - \psi$ mate with one randomly encountered male. ψ is independent of female genotype. It is also assumed that matings are not male limited, that is, the number of males does not influence the mating frequencies. The four monotypic matings (two female

genotypes can each mate with two male genotypes) and six additional polyandrous matings sum to 10 possible mating combinations (see Table 2). Mating frequencies, expressed as probabilities, can be calculated by multiplying the parental genotype frequencies. For example, the frequency of single matings between a female of genotype a and a male of genotype b can be expressed as

$$P(a) \cap P(b) = (1 - \psi) P_{a(1)} P_{b(2),} \quad (1)$$

where $a, b \in \{+ + \ , \ + t \ \}$.

The frequency for a multiple mating involving the ordered male genotypes b_1 and b_2 are given by

$$P(a) \cap P(b_1) \cap P(b_2) = \psi P_{a(1)} P_{b_1(2)} P_{b_2(2)}. \quad (2)$$

Note that equations (1) and (2) determine the probability for all possible matings, but they do not determine the outcome of the matings. This is done in the next section.

(2) After mating, the proportion of the different zygotes of the different crosses and double-crosses is calculated. We distinguish between within and between ejaculate effects.

Within ejaculate effects. Because meiotic drive changes the proportion of functional gametes in heterozygous males, the expected zygotes of the affected crosses deviate from Mendelian predictions. Segregation ratio (proportion of t gametes in the functional male gamete pool) in males is characterized by the variable $0 \leqslant \tau \leqslant 1$.

Between ejaculates effects. If a female mates with more than one male, both "quantity" and "quality" of the sperm are assumed to determine the fertilization success of the involved males (gamete fitness). The mating order of the males shall not have an influence on fertilization success. Quantity reduction, represented by the coefficient ν, results from the reduction of functional gametes through ratio distortion τ. Under the assumption that every male produces the same amount of sperm (functional and dysfunctional) per ejaculate, the *fraction* of functional sperm in a given ejaculate is dependent on TRD because the latter operates on rendering + sperm dysfunctional. In +/+ males that do not exhibit TRD, no sperm is lost and thus $\nu_{++} = 1$. In $+/t$ males, ν is dependent on the level of τ and can be expressed by $\nu_{+t}(\tau) = (1 + |2\tau - 1|)^{-1}$. The higher the deviation from $\tau = 0.5$, the bigger the individual males' loss in functional sperm. For $\tau > 0.5$, it can also be described as $\nu_{+t}(\tau) = 1/2\tau$. Because this expression summarizes the relevant cases for the t haplotype and is mathematically easier to handle, it will be used here.

Differences in quality between the two males ejaculates are modeled with the parameter c, which describes the relative disadvantage of the remaining heterozygote derived sperm. Hence, relative competitiveness of +/+ male sperm is $w_{++} = 1$, relative competitiveness of $+/t$ male sperm $w_{+t} = 1 - c$.

Given two males of genotype b_1, $b_2 \in \{+ +, + t \}$, the probability F_{b_1} of fertilization of a given egg by the male of genotype b_1 is dependent on both gamete fitness components w and ν and can be expressed by

$$F_{b_1} = \frac{w_{b_1} \nu_{b_1}}{w_{b_1} \nu_{b_1} + w_{b_2} \nu_{b_2}}. \quad (3)$$

If both males have the same genotype (e.g., $b_1 = b_2 =$ '$+ t$'), then $F_{b_1} = 0.5$. Note that $F_{b_1} + F_{b_2} = 1$. Knowing this, the proportions of zygote genotypes produced by each mating cross P_a^z can be determined (see Table 1 for one illustrative example and Table 2 for an overview over all possible mating crosses). The zygote frequencies of the total population $\bar{P}_{a(i)}^z$ of the genotypes $a \in \{+ +, + t, tt \}$ are then given by the sum of the outcomes of each individual mating cross P_a^z weighed by the frequency of the respective mating cross derived in equations (1) and (2).

(3) In the next step, viability selection occurs. The different genotypes are given different probabilities to establish themselves as adults in the population. Estimates from our wild population suggest that these probabilities can be sex dependent and eventually lead to sexually antagonistic effects. Relative viability differences between +/+ and $+/t$ individuals will be characterized by s_i, where i again defines sex. Overdominance is indicated

Table 1. One example showing how to calculate the zygote frequencies P_a^z of genotypes $a \in \{+ +, + t, tt\}$ for the multiple cross involving a female of genotype $a = + t$ and males of genotype $b_1 = ++$ and $b_2 = + t$. F_{b_1} and F_{b_2} are derived from equation (3). The zygote frequencies for all other crosses can be calculated likewise.

		Male b_1	**Male** b_2		
	Gametes	+	+	t	Total
Female a	+	$\frac{1}{2}F_{b_1}$	$\frac{1}{2}F_{b_2}(1-\tau)$	$\frac{1}{2}F_{b_2}\tau$	$\frac{1}{2}$
	t	$\frac{1}{2}F_{b_1}$	$\frac{1}{2}F_{b_2}(1-\tau)$	$\frac{1}{2}F_{b_2}\tau$	$\frac{1}{2}$
	Total	F_{b_1}	F_{b_2}		1
Resulting zygote frequencies (using eq. 3)					
$P_{+/+}^z = \frac{1}{2}F_{b_1} + \frac{1}{2}F_{b_2}(1-\tau) = \frac{1-c+\tau+\tau c}{2(1-c+2\tau)}$					
$P_{+/t}^z = \frac{1}{2}F_{b_1} + \frac{1}{2}F_{b_2}(1-\tau) + \frac{1}{2}F_{b_2}\tau = \frac{1}{2}$					
$P_{t/t}^z = \frac{1}{2}F_{b_2}\tau = \frac{\tau-\tau c}{2(1-c+2\tau)}$					

Table 2. The different possible single (female of genotype *a* mating with a male of genotype *b*) and multiple crosses (female of genotype *a* mating with males of genotypes b_1, b_2) in the polyandry model, including their resulting zygote proportions (P_a^z for $a \in \{+\,+, +\,t, tt\}$). Expected paternity shares at birth (correcting for *t*/*t* lethality) of the first male b_1, and the second male b_2, are given in the cases of multiple crosses.

		Zygote frequencies per mating cross			Paternity shares at birth	
		$P^z{}_{++}$	$P^z{}_{+t}$	P^z_{tt}	b_1	b_2
	+/t ∗ +/t	$\frac{1-\tau}{2}$	$\frac{1}{2}$	$\tau/2$		
Single matings	+/+ ∗ +/t	$\frac{1}{2}$	$\frac{1}{2}$	0		
male b × female a	+/t ∗ +/+	$1-\tau$	τ	0		
	+/+ ∗ +/+	1	0	0		
	(+/t, +/t) ∗ +/t	$\frac{1-\tau}{2}$	$\frac{1}{2}$	$\tau/2$	$\frac{1}{2}$	$\frac{1}{2}$
	(+/+, +/+) ∗ +/t	$\frac{1}{2}$	$\frac{1}{2}$	0	$\frac{1}{2}$	$\frac{1}{2}$
Multiple matings	(+/+, +/t) ∗ +/t	$\frac{1-c+\tau+\tau c}{2(1-c+2\tau)}$	$\frac{1}{2}$	$\frac{\tau-\tau c}{2(1-c+2\tau)}$	$\frac{4\tau}{2+3\tau-2c+\tau c}$	$\frac{2-\tau-2c+\tau c}{2+3\tau-2c+\tau c}$
(male b_1, male b_2) × female a	(+/+, +/+) ∗ +/+	1	0	0	$\frac{1}{2}$	$\frac{1}{2}$
	(+/t, +/t) ∗ +/+	$1-\tau$	τ	0	$\frac{1}{2}$	$\frac{1}{2}$
	(+/+, +/t) ∗ +/+	$\frac{1-c+\tau+\tau c}{1-c+2\tau}$	$\frac{\tau-\tau c}{1-c+2\tau}$	0	$\frac{2\tau}{1-c+2\tau}$	$\frac{1-c}{1-c+2\tau}$

by $s_i < 0$ and underdominance by $s_i > 0$. Thus, relative viabilities for $+/+$ genotypes are $w_{++(i)} = 1$ and $w_{+t(i)} = 1 - s_i$ for $+/t$ genotypes, respectively. Because the t haplotypes of our study population carry an embryonic lethal (A. K. Lindholm, unpubl. data), viability of homozygote t carriers is set to be $w_{tt(i)} = 0$. Adult genotype frequencies for any genotype $a \in \{+\,+, +\,t\}$ after viability selection are given by

$$P'_{a(i)} = \frac{w_{a(i)}\bar{P}^z_{a(i)}}{\sum w_{a(i)}\bar{P}^z_{a(i)}}. \quad (4)$$

We use a scenario in which $\psi = 0$, $c = 0$, and $s_i = 0$ as a null model. This is equivalent to the model by Bruck (1957), and is a case of dominance of the wild-type allele, because both $+/+$ and $+/t$ have the same fitness, whereas t/t are lethal.

THE STUDY POPULATION

All the data used in the present study originate from a free-living population inhabiting a barn near Zurich. The population was established in 2002 from 12 founder individuals (originating from two different capture sites close-by). On an area of 72 $\{m^2$, animals were given breeding opportunities (40 nest boxes) and ad libitum food and water (around eight drinking and feeding sites). Branches and shelves allowed mice to establish several territories in the building and freely enter and leave the barn through numerous openings. The population has been intensively monitored from inception for births and deaths, and tissue samples for genetical analyses have been taken from each pup and adult, as well as from individuals found dead. For the period between 2003 and summer 2008 used for the present study, 2177 pups were sampled at an age of 13–15 days. We found a rate of about 5% unsampled individuals among all captured adults during this study period. We thus sampled at least 95% of all pups born in the population.

GENOTYPING

To identify the *t* haplotype, the *Hba-ps4* locus — a marker containing a 16-bp *t* haplotype-specific insertion (Hammer et al. 1989) — was amplified and scored. Sexing of pups was performed by amplification of three different Y-specific microsatellite markers (Y8, Y12, and Y21; Hardouin et al. (2010) and M. Teschke, pers. comm.). Samples in which no Y marker amplified were scored as females (A. K. Lindholm, unpubl. data). Identification of dead and adult individuals was also carried out by matching multi-locus genotypes of the dead or adult individuals with those of the sampled pups at 21 unlinked microsatellite loci, allowing one mismatch (A. K. Lindholm, unpubl. data). As pups were sampled at an age of 13 days, birth date could be calculated. Using the date on which the dead body was found as date of death, longevity could be determined.

Results

PREDICTIONS BASED ON DETERMINISTIC MODEL

To investigate the potential impact of polyandry on the *t* frequency p_t, the life cycle described by equations (1–4) was repeated for 1000 generations. Independent of the initial parameter settings, p_t reached a steady-state equilibrium $\hat{p}_t$ after only a few generations. Figure 1 shows $\hat{p}_t$ dependent on multiple mating frequency ψ and heterozygote relative sperm competitiveness c. Segregation distortion was set to the level observed in our population at

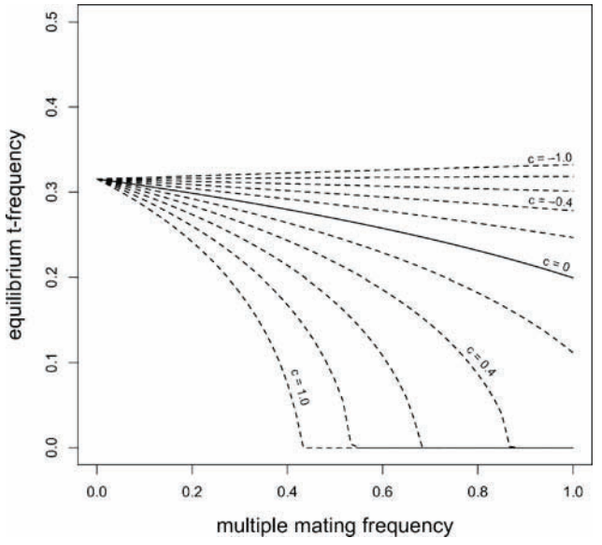

Figure 1. Equilibrium *t* frequency $\hat{p}_t$ dependent on multiple mating frequency (ψ) for different levels of *t* sperm competitiveness $-1 \leqslant c \leqslant 1$ and $\tau = 0.90$. The solid line represents the case where heterozygote males only suffer from quantitative sperm disadvantages resulting directly from segregation ratio distortion ($c = 0$).

$\tau = 0.90$ (see Supporting infor). If $+/t$ males have only a quantitative sperm competition disadvantage (lower viable sperm count) compared to $+/+$ males ($c = 0$, solid line in Fig. 1), polyandry does not have strong effects on *t* frequency. When all females mate multiply ($\psi = 1$), the proportion of $+/t$ individuals in a population is reduced by about 20% compared to the case where only single matings occur ($\psi = 0$). Frequency of matings involving a $+/t$ and a $+/+$ male—the cases where sperm competition disadvantage can play a role at all—is obviously too rare to produce substantial effects on $\hat{p}_t$. This changes however, if disadvantages in sperm quality are included in the quantitative sperm competition disadvantage of *t* carrying males ($c > 0$) . Higher disadvantages in $+/t$ sperm quality lead to lower equilibrium *t* frequencies. Depending on the level of *c*, effects on *t* frequency can be quite strong, especially when accompanied by high multiple mating rates.

PREDICTIONS BASED ON STOCHASTIC MODEL AND COMPARISON TO WILD HOUSE MOUSE POPULATION

Empirical observations. The model was applied to an intensively studied, free-living house mouse population near Zurich. A lethal version of the *t* haplotype was present in this population since its establishment. Figure 2A shows the frequency of the *t* over a 5.5-year time period (2003 until summer 2008) among 2177 pups. Although fluctuating substantially, *t* frequency decreased significantly over these five years (GLM on proportions using a binomial error structure and a logit link function using time as a continuous factor (in years); residual deviance: $\chi^2_{17} = 79.53$, $P < 0.001$).

The t paradox. This decrease is surprising given the substantial advantage for the *t* haplotype through segregation ratio distortion. For the levels of segregation ratio distortion found in the population ($\tau = 0.90$, see Supporting information for more details), Bruck (1957) predicts an equilibrium *t* frequency $\hat{p}_t$ as high as 0.32 (see Fig. 2A). In addition, we found that *t* heterozygote females have a significantly longer life expectancy than homozygote wild-type females (Cox proportional hazard model: $n = 174$, $\exp(\beta)$=2.46, $P < 0.01$, see Figure 3 and Supporting information for more details). Male survival on the other hand was not dependent on the *t* haplotype (Cox proportional hazard model: $n = 185$, $\exp(\beta)$=1.26, $P = 0.31$). The higher survival probabilities of female heterozygotes hence leads to overdominance (as compared to the Null model, which is a case of dominance). We used data from 21 unlinked microsatellite markers to examine inbreeding. Inbreeding levels did not change throughout the whole observation period. A linear regression analysis showed that the F_{IS} (deviations in heterozygosity from Hardy–Weinberg predictions) did not significantly change over time (Regression coefficients: $\alpha = 0.0259$, $\beta = -0.0065$, $n = 147$, $P = 0.07$, $r = 0.023$, see Fig. S1).

Polyandry. Based on paternity analysis, we find a proportion of litters sired by more than one male (multiple paternity rate) of around 0.3 (Camani 2005). Competitive ability *c* of $+/t$ derived sperm was estimated from laboratory experiments with wild mice (A. Manser, unpubl. data). Using an elaborate choice device that allowed females free physical access to both a $+/+$ and a $+/t$ male without male interference, we found a proportion of 0.19 (95% CI using a binomial error distribution: [0.08, 0.35]) sired by the $+/t$ male among four multiply sired litters. The experimental setup did not control for mating order. This would correspond to $c = 0.58$ (following Table 2). We are, however, facing a fundamental problem. Multiple paternity rate and sperm competitiveness of $c = 0.58$ mark only the "minimal" amount of sperm competition possible in our system. Cases of multiple matings with only one successful competitor do not lead to multiple paternity and are therefore missed by our analysis. The amount of such cases highly depends on sperm competitiveness *c*, the litter size λ as well as *t* frequency p_t in the population. But we cannot estimate both *c* and ψ only knowing multiple paternity rate. We are left with an underdefined system that forces us to make assumptions.

Scenario I: This most conservative scenario assumes that all multiple matings resulted in multiple paternities. This leaves us with a multiple mating frequency of $\psi = 0.3$ and a *t* sperm competitiveness of $c = 0.58$.

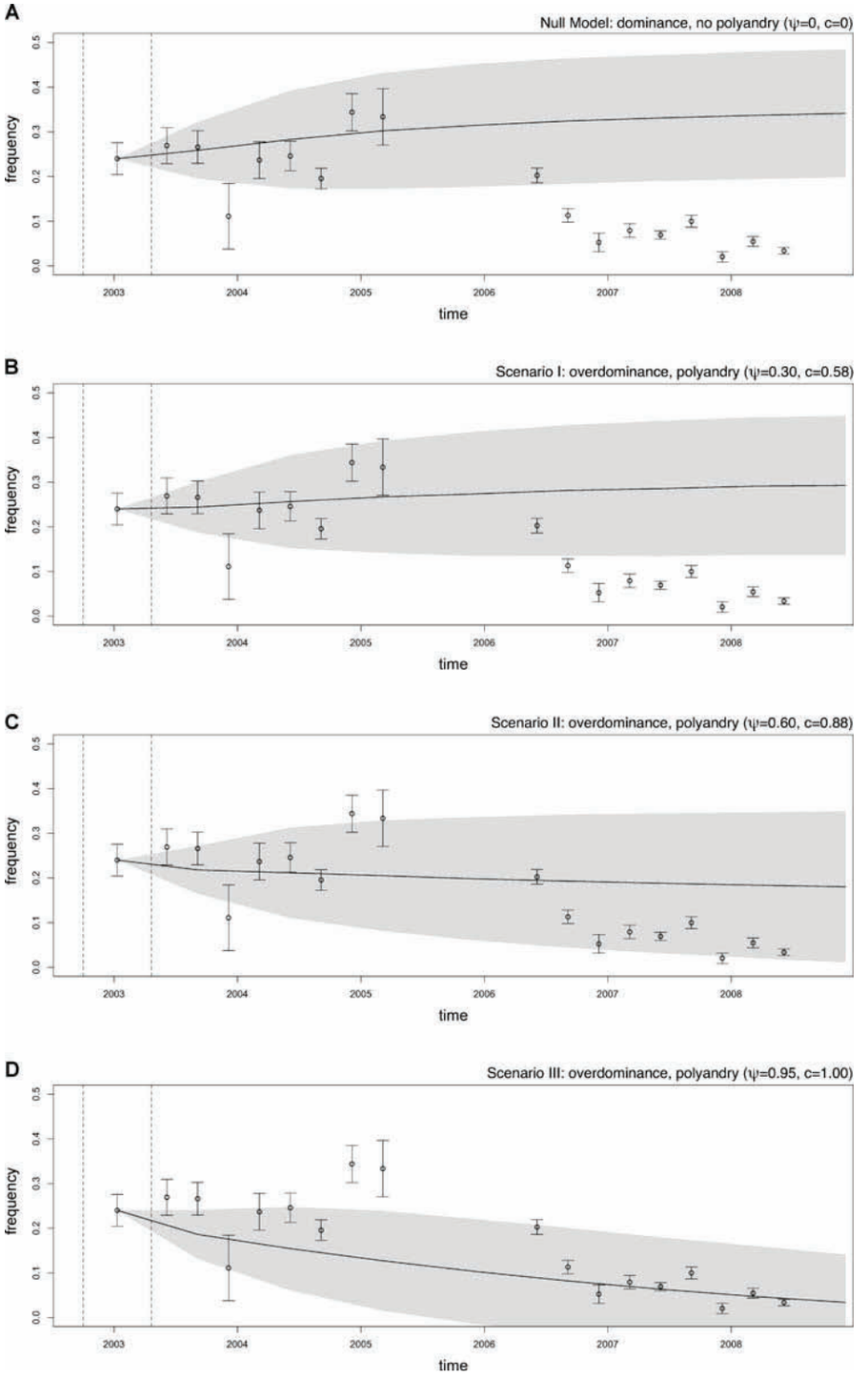

Figure 2. Observations from the wild population and simulation predictions from October 2002 until June 2008. The first dot within vertical dashed lines represents the initial *t* frequency among the first 50 adults ± SE. The following dots represent the *t* frequency among the pups born in the given 3 month time interval ± SE (n_{tot} = 2177). Solid lines show model predictions with 95%-confidence intervals (shadows) for (A) the null model (dominance and no polyandry) and (B)–(D) scenarios I–III (in Table 3, overdominance and different levels of polyandry). [Correction added June 1, 2011 after Online publication: Figure 2 legend updated to reflect black-and-white publication.]

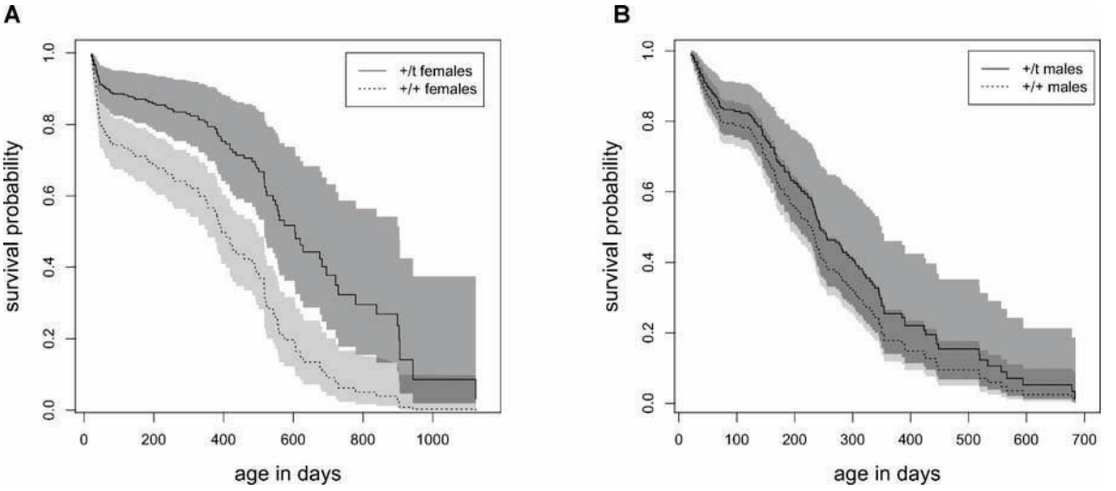

Figure 3. **Empirically estimated survival functions for (A) females and (B) males of different genetic backgrounds based on a Cox model. Each estimate is accompanied by a pointwise 95% confidence envelope.**

Scenario II: In this intermediate scenario, multiple mating frequency is assumed to be slightly higher. $\psi = 0.6$ is a value that is within the range of what has previously been described for wild house mouse populations (Dean et al. 2006). By making use of our model (with known litter size λ and mean t frequency $\bar{p}_t$) we find that $c = 0.88$ is needed to explain a gap between multiple paternity and multiple mating of this order.

Scenario III: The third scenario assumes an almost maximal multiple mating rate. It is based on the only study that actually looked at the mating behavior of females in an experimental context (Rolland et al. 2003). Given the choice between two different males, they found that 20 of 21 females (95%) mated with both of the males. Therefore, this scenario assumes $\psi = 0.95$ and $c = 1$.

Stochastic model. To determine if polyandry can explain this dramatic decrease in frequency, we ran simulations based on parameters estimated from the population (see Table 3 and Supporting information for a more detailed description). The deterministic, panmictic model described before was slightly modified. The deterministic nature of the model assuming infinite population size was now replaced by a stochastic simulation sampling randomly through the different life cycle stages based on effective population size and mean litter size. Life-history data from the populations allowed us to calculate an average time to reproduction (generation time) of 9 months (see Supporting information). This estimate was used to fit the simulation assuming nonoverlapping generations to the study population, where generations overlap. To get reliable estimates on model predictions and its confidence intervals, the 5.5-year observational period was simulated 10,000 times for each scenario. Initial frequency was set to the t frequency among the first 50 adults (equal to N_e, shown in red in Fig. 2, separated by a dotted line) which was $p_t = 0.24$.

Table 3. **Parameter estimates used in this study. Roman numerals refer to the three scenarios tested. Estimation of the lower part is shown in the Suppo.**

	Definitions	Estimates
ψ	Frequency of females mating twice	I: 0.30
		II: 0.60
		III: 0.95
c	Relative disadvantage of $+/t$ derived sperm	I: 0.58
		II: 0.88
		III: 1.00
λ	Average litter size at birth	5.47
γ	Generation time	9 months
N_e	Effective population size	50
τ	Segregation ratio distortion	0.90
s_1	Difference in survival between $+/+$ females and $+/t$ females	−0.30
s_2	Difference in survival between $+/+$ males and $+/t$ males	0.00

Figure 2A shows the stochastic model predictions for the null model (following Bruck (1957)), which assumes no polyandry, equal fitness between $+/+$ and $+/t$ individuals, and homozygote t/t lethality (dominance). Figure 2B–D shows the three different polyandry scenarios that include female overdominance and sperm competition. Scenarios 2 and 3 with high polyandry fit the

data considerably better than the null model. Notice that—in contrast to scenario 2—the deterministic equilibrium prediction for scenario 3 is the extinction of the *t*. However, more generations than used in the present simulation are on average needed to reach extinction.

EFFECTS ON MEAN POPULATION FITNESS

Examining mean fitness of the overall population here reveals interesting effects of the *t* haplotype. As is the case for all selfish genetic elements (Burt and Trivers 2006), the *t* haplotype elicits conflict between different levels of selection (allelic propagation versus population fitness). This conflict can be revealed when looking at the mean population fitness and its related gene frequencies. In the case of overdominance, mean population fitness is generally optimized by maximizing the number of heterozygotes in a population (Wright 1929). However, in the presence of distorters, the expectation that Mendelian segregation and random mating lead to gene frequency changes that increase mean population fitness (Wright 1929) no longer holds. By increasing their own frequency, *t* haplotypes drag the population frequency away from the optimum. This is shown in Figure 4 for our specific case: in accordance with Wright, without segregation distortion (solid line) an equilibrium frequency that optimizes population fitness is reached (point [1]). As soon as we add meiotic drive to the system (dashed line), mean population fitness at equilibrium is far from the optimum (point [2]), manifesting the conflict between gene and population level fitness. Dependent on the scenario, these population fitness losses are recovered by the process of sperm competition. Whereas scenario 2 has an equilibrium *t* frequency that nearly corresponds to the optimum of population fitness, the deterministic model predicts extinction of the *t* and suboptimal population fitness in scenario 3. The observed *t* frequency at the end of the observation period (shown as vertical dash-dotted line) falls between maximum population fitness and extinction of the *t* haplotype.

Discussion

t dynamics. One of the main findings of this study is the significant decrease in *t* frequency in a wild population over a time period of five years. It is remarkable how strong the dynamics of the *t* haplotype in our study population closely resemble the patterns observed in other studies that examined *t* frequencies in a natural or seminatural context. Both Ardlie and Silver (1998) and Carroll et al. (2004) find low frequencies in their study populations of house mice, the latter report a similar decrease in frequency over time in their enclosure populations. Even in a different subspecies *(Mus castaneus)* in Taiwan, *t* frequencies are found at persistent but low frequencies (Huang et al. 2001). These striking parallels among studies measuring *t* frequencies in different geographic and phylogenetic contexts suggest similar general mechanisms. It also confirms our assumption that if we are able to unravel the factors determining the *t* dynamics in our specific population, these findings have implications for house mouse populations in general.

Parallels to other studies are not only restricted to *t* frequency dynamics, as most other parameter estimates confirm what has previously been found. TRD of 0.90 is a level typically observed in other natural and semi-natural populations (Ardlie and Silver 1996; Huang et al. 2001; Carroll et al. 2004), and provides no indication of a reduction of distortion level by modifiers (Ardlie 1998). The estimates concerning polyandry in our study population are also consistent with previous investigations. A marked multiple paternity rate of about 30% (Camani 2005) provides further support that house mice mating systems are highly polygynandrous. This estimate is further in high accordance with recent work, that found multiple paternity rates of 23% and 26% (Dean et al. 2006; Firman and Simmons 2008a).

Competitiveness of the $+/t$ derived sperm c is clearly the model parameter with the weakest support, both in our study and in the literature. Two other studies measured $+/t$ male paternity shares of 0.17 and 0.22 (Olds-Clarke and Peitz 1986; Ardlie and Silver 1996), proportions that are comparable to what has been found here (0.19). They contradict however slightly with Carroll et al. (2004) who found a paternity share for $+/t$ males of 0.36, a value that would suggest no "qualitative" differences in sperm (for $c = 0$ and $\tau = 0.90$ our model predicts a $+/t$ heterozygotes paternity share of $F_{+t} = 0.28$). Better estimates for this key parameter of the model are clearly needed to further support the polyandry hypothesis.

Female fitness advantage. $+/t$ females in our population have a longer life expectancy, which is in marked contrast to what has previously been reported. An early study found similar fitness advantages in both sexes (Dunn et al. 1958), but most recent publications show a different picture. In a detailed study on the fitness consequences of the *t*, Carroll et al. (2004) find fitness disadvantages for *t* carriers both in males and females (both in survivorship and reproductive output). They argue further that artificially high levels of competition in their enclosure populations might amplify these differences. Indeed, we find indicators of high competition such as wounded adult males and females, and dead pups inside and outside nest boxes (presumably killed by conspecifics) in our study population—apparently not only among males, but surprisingly also among females. The fact that in contrast to the study of Carroll et al. (2004), individuals always had the opportunity to leave the population, makes this even more surprising. Reproductive skew turned out to be strong both in males and females (A. K. Lindholm, unpubl. data). Furthermore, more pups are produced each year than are able to recruit into the population (see Supporting information). However, we have

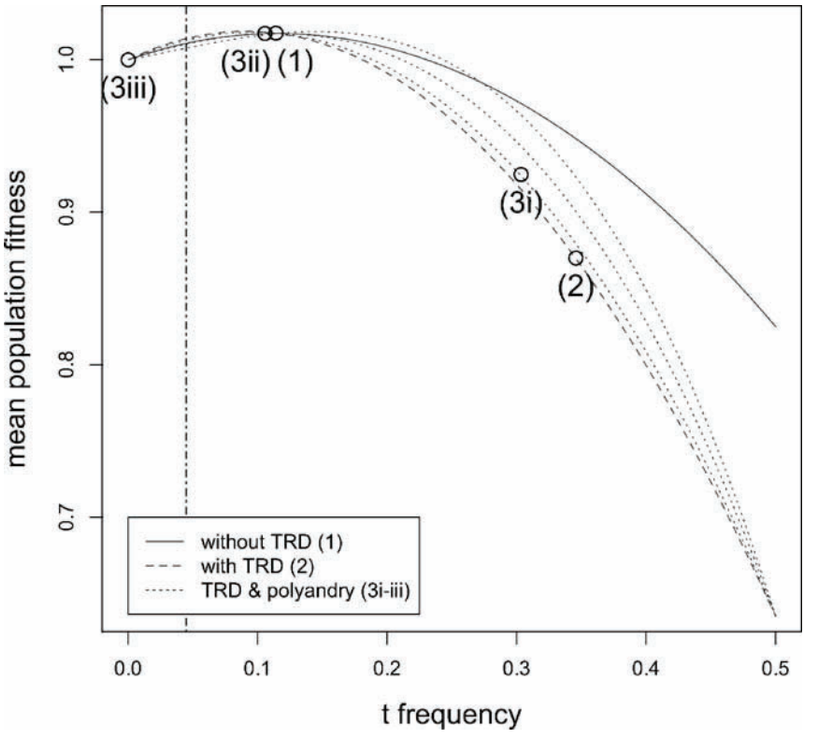

Figure 4. **Mean population fitness for the offspring generation as a function of the p_t of the parental generation (assuming 1:1 male to female ratio) and their associated equilibrium frequencies (dots) for no TRD and polyandry (solid gray line and point (1)), TRD without polyandry (dashed gray line and point (2)), and the complete models with TRD and polyandry (dotted gray lines and point (3i–iii) corresponding to scenarios I–III). The vertical line with dash-points corresponds to observed *t* frequency in the population in summer 2008 (GLM prediction: $p_t = 0.045$).**

no information about what happens to mice that disperse out of the population. Our longevity estimates are based on nondispersing mice out of the barn, thus our result of genotype-dependent survival makes the assumption that there is no differential emigration. Even if our survival data are confounded with migration, it nevertheless seemed to be appropriate to use the longevity estimates for the model, because they provide reliable information about the probability of an individual to establish in the population of interest.

The conflict between different levels of selection. The implications of the combination of fitness effects between individual and mean population fitness are intriguing. Weak overall overdominance results if female positive and male neutral effects are merged together (see Fig. 4). It follows from this that maximum population fitness is reached if a considerable number of *t* haplotypes are still present in the population. This finding is counterintuitive, because distorter systems are usually characterized as "genomic parasites." In this case however, a certain amount of parasites is beneficial to the collective. This would suggest that there is an intermediate optimal rate of polyandry, as Arnqvist and Nilsson (2000) suggest in a meta-analysis on insects. Nevertheless, negative effects are still severe if two parasites occur in the same individual (t/t homozygosity). Meiotic drive makes these cases more frequent. The relation between equilibrium gene frequency and population fitness (Fig. 4) illustrates how nonrandom segregation can lead to outcomes that are no longer maximizing individual or population fitness. Sexual selection in the form of polyandry and sperm competition is a possible mechanism to recover losses in individual and population fitness by reducing the number of t/t zygotes produced. Hence, multiple mating can be seen as an adaptive strategy for the organism to increase individual fitness. The question however, if an internal *t* frequency equilibrium optimizing mean population fitness is reached, depends on the scenario. The observed *t* frequency dynamics do not offer a conclusive answer to this question, because *t* frequency at the end of the 5.5-year period falls between the population fitness optimum and *t* extinction.

The conflict between levels of selection can also be seen as a conflict among the genes. Nonlinked genes can only spread

through populations when they increase individual fitness (as is the case if mean population fitness is maximized). In this respect, polyandry would represent an indirect solution to intragenomic conflict: by building up organisms that pursue a polyandrous mating strategy, unlinked genes are able to prevent *t* haplotypes from reaching frequencies that are too high. However, the overall overdominance effect is only weak and appears to be rather specific for the observed population. Thus, maximization of population fitness may not provide a general answer to the question of why *t* haplotypes generally occur at small but stable frequencies.

The model predictions. Our specific case study provides a further example that the null model provided by Bruck (1957)—even when allowing for drift—is not sufficient to explain the naturally observed frequencies. In contrast to the null model, our model shows that polyandry in the context of a meiotic drive system is a potential mechanism to explain the low *t* frequency paradox.

There are plenty of reasons to assume that sperm competition levels are substantially higher than assumed in scenario I. First, the assumption of a maximal number of two matings per female is clearly a simplification. Rolland et al. (2003), for example, showed that females can regularly change mating partners during one estrous cycle (13 out of 21 females received three ejaculations from two potential mating partners during one estrous cycle). The probability p of having *at least* one *t* carrying male among all males mated increases with every mating partner. If k is the number of partners per estrous cycle and $P_{+t(2)}$ the proportion of heterozygote males in a population, this probability increases with k given $p = (1 - P_{+t(2)})^k$. This effect would increase the number of matings where the $+/+$ sperm competition advantage plays a role. Second, we only looked at competitiveness effects dependent on the donor's genotype. This is of course a simplification, because other factors such as mating order usually have a major impact on reproductive success. The integration of such factors would not change our predictions of *t* frequencies (assuming that the mating order remains random). However, it would strongly increase the discrepancy between multiple matings and the actually measured multiple paternities. This stresses once more the urgency to estimate the specific factors determining male reproductive success in sperm competition in the future.

Obviously, there is still the possibility that the forces included in the present model are insufficient to explain the observed *t* dynamics. However, most of the potential factors that have been proposed in the last 50 years have been considered here. Population substructure appears rather unlikely given that migration within the barn is clearly too high to result in different demes. Even if there was a certain amount of substructure in the barn, Levin et al. (1969) showed that only small migration rates are needed to counterbalance the loss of *t* haplotypes by genetic drift as described in Lewontin and Dunn (1960). Inbreeding (Petras and Topping 1983) was excluded here as a possible factor (see Fig. S4). Migration of *t* carriers into the population is unlikely to affect *t* dynamics: We only found 5% unmarked adults (see Material and Methods) and genetic analyses did not identify immigrants among them (A. K. Lindholm, unpubl. data). There is also the possibility that precopulatory female mating bias interacts with the here described postcopulatory effects of sperm competition. At first sight, one might think that these two hypotheses could be exclusive. Why should females mate with multiple partners if they can recognize and discriminate between the different genotypes (as indeed suggested by Coopersmith and Lenington (1992))? However, female mating decisions might not always be as free and unconstrained as assumed. If the dominant male of their territory is a *t* carrier, females may not have any choice but to mate with him or risk infanticide (Perrigo et al. 1991). Results by Coopersmith and Lenington (1992) indicate that male dominance status is rated higher by females than genetic background. If one would assume that this preference is adaptive, this result would indicate that the costs for a female to mate exclusively with a subordinate male are even higher than the costs of mating with a $+/t$ male. However, the correlation between male dominance and the *t* genotype is unclear. In arena experiments, $+/t$ males tended to dominate $+/+$ males (Lenington et al. 1996), whereas Carroll et al. (2004) found $+/+$ males to be dominant in a seminatural context. In any case, an interaction of both multiple mating and precopulatory mate choice could definitely have more profound effects on equilibrium *t* frequency. It would be very interesting to investigate this further. For example, one might expect an increase in polyandry in the case of many *t* carrying males present in a population, as Price et al. (2008b) showed in *D. pseudoobscura*.

General implications. Many have wondered why one cannot find modifiers in the genome that fight *t* distortion on a genetic level in naturally occurring house mouse populations (Ardlie 1998). The previously described X-linked *SR* system in *D. pseudoobscura* is one of the other cases where no modifiers have been found so far (Beckenbach 1996). The results presented here and those of Beckenbach (1996) and Ardlie (1998) suggest an alternative scenario: the possibility that there was never a need for such suppressors to evolve, because the *t* haplotype and the *SR* were already facing severe problems to be successful in polygynandrous mating systems. Thus, the mechanisms that such genetic elements use to make them strong intraejaculate competitors (reduced number of + sperm) can at the same time make them vulnerable to sperm competition, and hence, multiple mating. Individual behavior in the form of female mating decisions would in this case improve individual fitness (to the disadvantage of the *t*). Alternatively, the genes determining multiple mating could be regarded as another class of genetic modifiers of segregation distortion.

The results presented here demonstrate that polyandry is a biologically plausible explanation for the low *t* frequency paradox.

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LITERATURE CITED

Ardlie, K. 1998. Putting the brake on drive: meiotic drive of *t* haplotypes in natural populations of mice. Trends Genet. 14:189–193.

Ardlie, K., and L. Silver. 1996. Low frequency of mouse *t* haplotypes in wild populations is not explained by modifiers of meiotic drive. Genetics 144:1787–1797.

———. 1998. Low frequency of *t* haplotypes in natural populations of house mice *(Mus musculus domesticus)*. Evolution 52:1185–1196.

Arnqvist, G., and T. Nilsson. 2000. The evolution of polyandry: multiple mating and female fitness in insects. Anim. Behav. 60:145–164.

Artzt, K., P. McCormick, and D. Bennett. 1982. Gene mapping within the *T*/*t* complex of the mouse. I: *t*-lethal genes are nonallelic. Cell 28: 463–470.

Atlan, A., C. Capillon, N. Derome, D. Couvet, and C. Montchamp-Moreau. 2003. The evolution of autosomal suppressors of sex-ratio drive in *Drosophila simulans*. Genetica 117:47–58.

Atlan, A., D. Joly, C. Capillon, and C. Montchamp-Moreau. 2004. Sex-ratio distorter of *Drosophila simulans* reduces male productivity and sperm competition ability. J. Evol. Biol. 17:744.

Bauer, H., N. Véron, J. Willert, and B. Herrmann. 2007. The *t*-complex-encoded guanine nucleotide exchange factor *Fgd2* reveals that two opposing signaling pathways promote transmission ratio distortion in the mouse. Genes Develop. 21:143.

Bauer, H., J. Willert, B. Koschorz, and B. Herrmann. 2005. The *t* complex-encoded GTPase-activating protein *Tagap1* acts as a transmission ratio distorter in mice. Na. Genet. 37:969–973.

Beckenbach, A. 1996. Selection and the 'sex-ratio' polymorphism in natural populations of *Drosophila pseudoobscura*. Evolution 50:787–794.

Bennett, D., A. Alton, and K. Artzt. 1983. Genetic analysis of transmission ratio distortion by *t*-haplotypes in the mouse. Genet. Res. 41:29.

Bruck, D. 1957. Male segregation ratio advantage as a factor in maintaining lethal alleles in wild populations of house mice. Proc. Natl. Acad. Sci. USA 43:152–158.

Burt, A., and R. Trivers. 2006. Genes in conflict: the biology of selfish genetic elements. Belknap Press, Cambridge.

Camani, M. 2005. Genetic evidence of multiple paternity in a wild house mouse population. Master's thesis, University of Zurich, Zurich.

Carroll, L., S. Meagher, L. Morrison, D. Penn, and W. Potts. 2004. Fitness effects of a selfish gene (the *Mus t* complex) are revealed in an ecological context. Evolution 58:1318–1328.

Charlesworth, B., and D. Hartl. 1978. Population dynamics of the segregation distorter polymorphism of *Drosophila melanogaster*. Genetics 89:171–192.

Coopersmith, C., and S. Lenington. 1992. Female preferences based on male quality in house mice: interaction between male dominance rank and *t*-complex genotype. Ethology 90:1–16.

Champion de Crespigny, F., and N. Wedell. 2006. *Wolbachia* infection reduces sperm competitive ability in an insect. Proc. R. Soc. Lond. B 273:1455–1458.

Dean, M., K. Ardlie, and M. Nachman. 2006. The frequency of multiple paternity suggests that sperm competition is common in house mice *(Mus domesticus)*. Mol. Ecol. 15:4141–4151.

Dobrovolskaia-Zavadskaia, N., and N. Kobozieff. 1927. Sur la reproduction des souris anoures. Comptes Rendus Séances Société de Biologie et de ses Filiales 97:116–119.

Dod, B., C. Litel, P. Makoundou, A. Orth, and P. Boursot. 2003. Identification and characterization of *t* haplotypes in wild mice populations using molecular markers. Genet. Res. 81:103–114.

Dunn, L., A. Beasley, and H. Tinker. 1958. Relative fitness of wild house mice heterozygous for a lethal allele. Am. Nat. 92:215–220.

Firman, R., and L. Simmons. 2008a. The frequency of multiple paternity predicts variation in testes size among island populations of house mice. J. Evol. Biol. 21:1524–1535.

———. 2008b. Polyandry facilitates postcopulatory inbreeding avoidance in house mice. Evolution 62:603–611.

———. 2008c. Polyandry, sperm competition, and reproductive success in mice. Behav. Ecol. 19:695–702.

Gummere, G., P. McCormick, and D. Bennett. 1986. The influence of genetic background and the homologous chromosome 17 on *t*-haplotype transmission ratio distortion in mice. Genetics 114:235–245.

Haig, D., and C. Bergstrom. 1995. Multiple mating, sperm competition and meiotic drive. J. Evol. Biol. 8:265–282.

Hammer, M., J. Schimenti, and L. Silver. 1989. Evolution of mouse chromosome 17 and the origin of inversions associated with *t* haplotypes. Proc. Natl. Acad. Sci. USA 86:3261–3265.

Hardouin, E. A., J. L. Chapuis, M. I. Stevens, J. B. van Vuuren, P. Quillfeldt, R. J. Scavetta, M. Teschke, and D. Tautz. 2010. House mouse colonization patterns on the sub-Antarctic Kerguelen Archipelago suggest singular primary invasions and resilience against re-invasion. BMC Evol. Biol. 10:325.

Hartl, D. 1970. A mathematical model for recessive lethal segregation distorters with differential viabilities in the sexes. Genetics 66:147–163.

Hiraizumi, Y., and A. Thomas. 1984. Suppressor systems of segregation distorter *(SD)* chromosomes in natural populations of *Drosophila melanogaster*. Genetics 106:279–292.

Huang, S. W., K. G. Ardlie, and H. T. Yu. 2001. Frequency and distribution of *t*-haplotypes in the Southeast Asian house mouse *(Mus musculus castaneus)* in Taiwan. Mol. Ecol. 10:2349–2354.

Johnston, P., and G. Brown. 1969. A comparison of the relative fitness of genotypes segregating for the t^{w2} allele in laboratory stock and its possible effect on gene frequency in mouse populations. Am. Nat. 103:5–21.

Klein, J., P. Sipos, and F. Figueroa. 1984. Polymorphism of *t*-complex genes in European wild mice. Genet. Res. 44:39–46.

Lenington, S., C. Coopersmith, and M. Erhart. 1994. Female preference and variability among *t*-haplotypes in wild house mice. Am. Nat. 143:766–784.

Lenington, S., L. Drickamer, A. Robinson, and M. Erhart. 1996. Genetic basis for male aggression and survivorship in wild house mice *(Mus domesticus)*. Aggressive Behav. 22:135–145.

Lenington, S., and K. Egid. 1985. Female discrimination of male odors correlated with male genotype at the *T* locus: a response to *T*-locus or *H-2*-locus variability? Behav. Genet. 15:53–67.

Lenington, S., P. Franks, and J. Williams. 1988. Distribution of t-haplotypes in natural populations of wild house mice. J. Mammal. 69:489–499.

Levin, B., M. Petras, and D. Rasmussen. 1969. The effect of migration on the maintenance of a lethal polymorphism in the house mouse. Am. Nat. 103:647–661.

Lewontin, R. 1968. The effect of differential viability on the population dynamics of *t* alleles in the house mouse. Evolution 22:262–273.

Lewontin, R., and L. Dunn. 1960. The evolutionary dynamics of a polymorphism in the house mouse. Genetics 45:705–722.

Lyon, M. 2003. Transmission ratio distortion in mice. Annu. Rev. Genet. 37:393–408.

Nunney, L. 1993. The role of deme size, reproductive patterns, and dispersal in the dynamics of *t*-lethal haplotypes. Evolution 47:1342–1359.

Okasha, S. 2006. Evolution and the levels of selection. Oxford Univ. Press, Oxford.

Olds-Clarke, P., and B. Peitz. 1986. Fertility of sperm from *t*/+ mice: evidence that +-bearing sperm are dysfunctional. Genet. Res. 47:49–52.

Perrigo, G., L. Belvin, and F. Vom Saal. 1991. Individual variation in the neural timing of infanticide and parental behavior in male house mice. Physiol. Behav. 50:287–296.

Petras, M., and J. Topping. 1983. The maintenance of polymorphisms at two loci in house mouse *(Mus musculus)* populations. Genome 25: 190–201.

Price, T., A. Bretman, T. Avent, R. Snook, G. Hurst, and N. Wedell, 2008a. Sex ratio distorter reduces sperm competitive ability in an insect. Evolution 62:1644–1652.

Price, T., D. Hodgson, Z. Lewis, G. Hurst, and N. Wedell. 2008b. Selfish genetic elements promote polyandry in a fly. Science 332:1241–1243.

Price, T., and N. Wedell. 2008. Selfish genetic elements and sexual selection: their impact on male fertility. Genetica 134:99–111.

Rolland, C., D. MacDonald, and M. Berdoy. 2003. Free female choice in house mice: leaving best for last. Behaviour 140:1371–1388.

Schimenti, J. 2000. Segregation distortion of mouse *t* haplotypes the molecular basis emerges. Trends Genet. 16:240–243.

Silver, L. 1993. The peculiar journey of a selfish chromosome: mouse *t* haplotypes and meiotic drive. Trends Genet. 9:250.

Silver, L., and P. Olds-Clarke. 1984. Transmission ratio distortion of mouse *t* haplotypes is not a consequence of wild-type sperm degeneration. Develop. Biol. 105:250–252.

Snook, R., S. Cleland, M. Wolfner, and T. Karr. 2000. Offsetting effects of *Wolbachia* infection and heat shock on sperm production in *drosophila simulans* analyses of fecundity, fertility and accessory gland proteins. Genetics 155:167–178.

Wright, S. 1929. Fisher's theory of dominance. Am. Nat. 68:562–565.

Young, S. 1967. A proposition on the population dynamics of the sterile *t* alleles in the house mouse. Evolution 21:190–192.

Zeh, J., and D. Zeh. 1996. The evolution of polyandry I: intragenomic conflict and genetic incompatibility. Proc. R. Soc. Lond. B 263:1711–1717.

———. 1997. The evolution of polyandry II: post-copulatory defences against genetic incompatibility. Proc. R. Soc. Lond. B 264:69–75.

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Supporting Information

The following supporting information is available for this article:

Text S1. Parameter Estimation
Figure S1. Inbreeding.
Figure S2. Pup survival.
Figure S3. Overall survival and fertility curves.

Supporting Information may be found in the online version of this article.

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Supporting Information

Text S1 — Parameter Estimation

γ, λ — **Generation time, Average litter size at birth (Life history parameters).** To get a reliable estimate for generation time (the average time to reproduction) both overall survival and fertility dependent on age were used (l_x and m_x curves).

Survival. Since genetic sampling is only possible after an age of 13 days, newborns can not be followed individually before the day of sampling and therefore require a separate analysis. Therefore, survival estimation was divided into two parts: pup survival (until an age of 15 days, equivalent to weaning age) and adult survival (from 15 days onwards).

A comparison in litter size between the date of first discovery and the date of genetical sampling (usually around day 13) can be used to get an estimate for pup survival. For a total of 217 litters, information about its size when first found and at day of genetical sampling was available. Litter size decreased significantly with time (linear regression, $n = 434$, $p < 0.001$, $r = 0.07$) with an average loss of 0.14 pups per day (see Figure S2). The regression model predicts an average litter size at birth of 5.47. To get an estimate for adult survival, we used individuals which had known birth and death date and which lived longer than 15 days for the analysis that were not necessarily part of the pup survival analysis.

Both pup and adult survival were integrated to estimate the whole survival curve (l_x curve, probability to survive into age class x). Average loss in litter size during the first 15 days (pups survival) was used to estimate the original number of newborns giving rise to the 267 individuals available for adult survival. The resulting Kaplan-Meier plot using a bin size of 50 days is shown in Figure S3A. Average life expectancy was 196 days.

Fertility. In order to get reliable estimates of net reproductive rate (R_0) and generation time (γ), only the 267 individuals where information about survival were known were included in the fertility analysis. Parentage information and the birth dates of the parents and the offspring

was available in 1258 cases. Figure S3B shows the average number of offspring produced in the different age classes (m_x curve).

Net reproductive rate R_0. The average amount of offspring produced by each individual (net reproductive rate R_0) can be determined from the survival and fertility curves and is simply $\sum l_x m_x$. Using this formula, we get a total R_0 of 1.12. This roughly corresponds to an intrinsic growth rate of $r = 0.16$ per year. This increase could be compensated by emigration (since population size is observed to be more or less constant).

Generation time. If survivorship l_x is defined as the probability to survive from birth to age class x and fecundity m_x is the number of offspring born to the parent of age class x generation time is

$$\gamma = \frac{\sum x l_x m_x}{\sum l_x m_x}.$$

γ can therefore be seen as a weighted mean at which any individual in the population had offspring, just as defined above. Using these survival and fertility distributions and an age-class-size of 50 days, total generation time was $\gamma = 263$ days.

N_e — **Effective Population Size.** In 2004, an average of 46 sexually mature adults were present in the population (Lindholm, unpublished). For our model, we assume an average population size of 50.

τ — **Transmission ratio distortion.** Among the twelve founder individuals, four were heterozygote t carriers. Absence of t/t homozygotes among all 2190 pups support the idea that there is only one version of the t haplotype occurring in the study population. Transmission ratio distortion (TRD) was estimated as 0.90 in controlled laboratory crosses using captured mice from the study population and their lab-born descendants (Lindholm, unpublished). This value strongly deviates from Mendelian expectations ($\chi^2_1 = 110.41, P < 0.001$). t frequency over the whole time period among all pups was $\bar{p_t} = 0.11$.

s_1, s_2 — **Differences in survival between +/+ and +/t individuals.** Survival depending on ge-

netic background was only analysed for individuals that lived longer than weaning (age of 20 days, Koenig and Markl [1987]). Pup viability was excluded for reasons of data quality. For 187 males and 171 females living longer than 20 days, information about the date of birth and death (i.e. longevity) were available. Further, information about reproductive activity (i.e. age at last reproduction) was used for an additional 114 females and 103 males as censored data. To determine possible differences in survival dependent on the genotype, we performed a Cox proportional hazard model (see Figure 2). The model did not find significant differences in longevity for males ($n = 185$, $\exp(\beta)$=1.26, $P = 0.31$). For females however, survival seems to be strongly influenced by the genetic background ($n = 174$, $\exp(\beta)$=2.46, $P < 0.01$). Both models met the proportional hazards assumption.

Knowing baseline pup survival (see Text S2) and the estimates for survival from the Cox model (see Figure 2), we can calculate the fraction of individuals still present at the typical time of reproduction (i.e. generation time γ). Doing so leaves us with 16.87% of all born +/+ females and 21.97% $+/t$ females (resulting in $s_1 = -0.30$). On the male side, 9.98% +/+ and 12.2% $+/t$ males remain at generation time γ. Since this difference was not significant, it was not included into the model, thus $s_2 = 0$.

Figure S1 — Inbreeding.

Figure S2 — Pup survival.

Figure S3 — Overall survival and fertility curves.

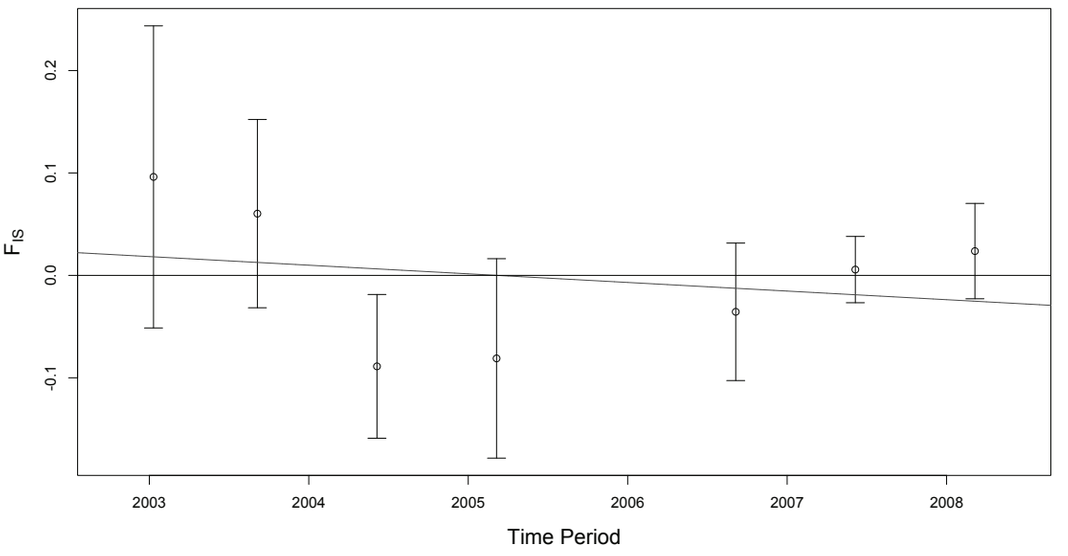

Figure 1. S4 — F_{IS} values (deviations from Hardy-Weinberg predictions) with standard deviations for 9 month time periods (identical the ones used in the model) with standard deviations. The grey line shows the linear regression line ($n = 147$, $P = 0.07$, $r = 0.023$) and the grey line the Hardy-Weinberg predictions.

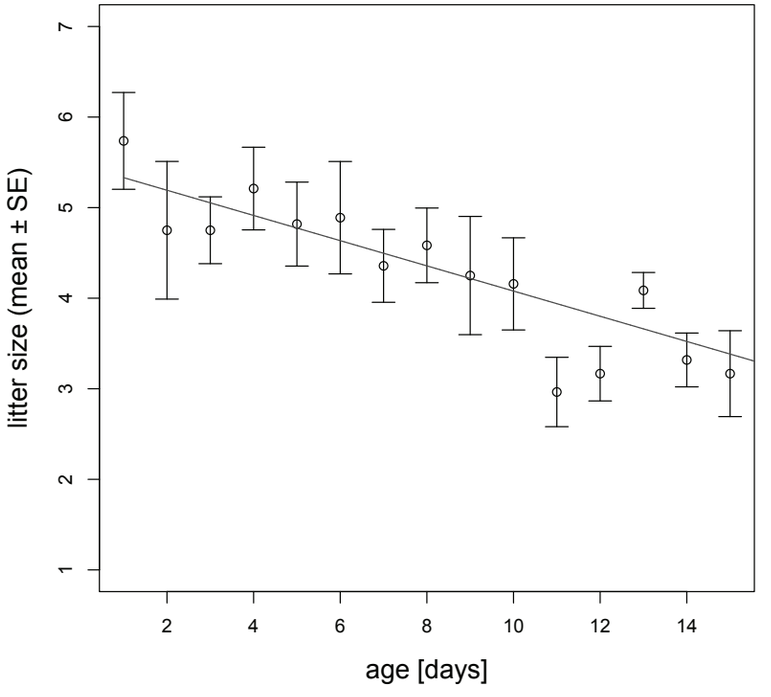

Figure 2. S2 — Average litter size with standard errors dependent on age of the litter with linear regression prediction (grey line, $n = 434$, $p < 0.001$, $r = 0.07$).

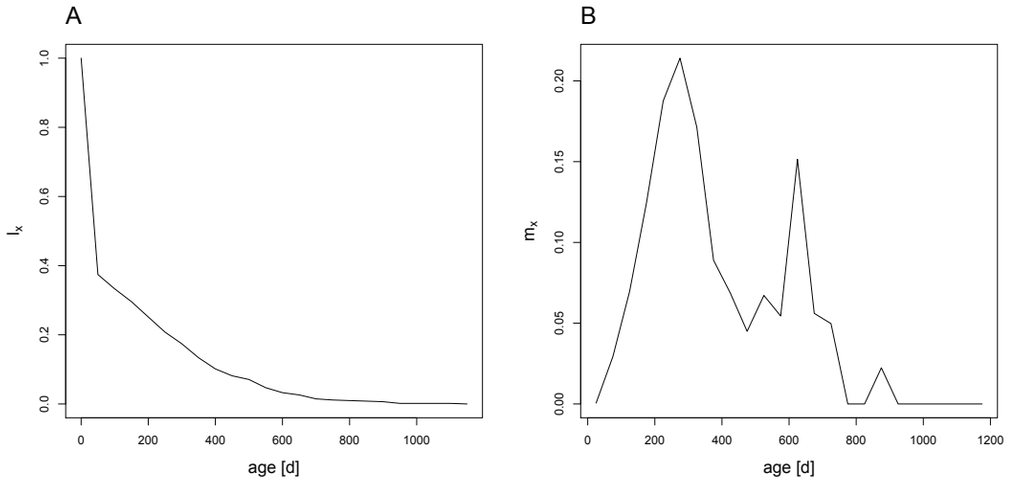

Figure 3. S3 — (A) Survival curves (Kaplan-Meier estimates) for males and females based on an age-class-size of 50 days. (B) Average number of offspring produced in these age classes. The three maxima seem to correspond to different years, suggesting seasonality in fertility.

Chapter 12

A selfish genetic element influencing longevity correlates with reactive behavioural traits in female house mice (*Mus domesticus*)

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A Selfish Genetic Element Influencing Longevity Correlates with Reactive Behavioural Traits in Female House Mice (*Mus domesticus*)

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Abstract

According to theory in life-history and animal personality, individuals with high fitness expectations should be risk-averse, while individuals with low fitness expectations should be more bold. In female house mice, a selfish genetic element, the *t* haplotype, is associated with increased longevity under natural conditions, representing an appropriate case study to investigate this recent theory empirically. Following theory, females heterozygous for the *t* haplotype (+/*t*) are hypothesised to express more reactive personality traits and be more shy, less explorative and less active compared to the shorter-lived homozygous wildtype females (+/+). As males of different haplotype do not differ in survival, no similar pattern is expected. We tested these predictions by quantifying boldness, exploration, activity, and energetic intake in both +/*t* and +/+ mice. +/*t* females, unlike +/+ ones, expressed some reactive-like personality traits: +/*t* females were less active, less prone to form an exploratory routine and tended to ingest less food. Taken together these results suggest that differences in animal personality may contribute to the survival advantage observed in +/*t* females but fail to provide full empirical support for recent theory.

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Introduction

In a wide range of taxa, it has been shown that individuals from the same population differ consistently in their behaviour. The concept of animal personality applies to behavioural differences that are consistent through time and situations [1,2,3]. Often, these behavioural traits are correlated within or across contexts and are referred to as behavioural syndromes [4,5,6]. For instance, "proactive" individuals, in contrast to "reactive" individuals, have higher activity levels and a higher metabolic rate, are more exploratory and risk-prone (or bold), and faster to establish routines [7,8,9,10,11]. How animal personalities evolved within populations still remains unclear, especially because behavioural plasticity could be seen as an optimal way to cope with fluctuating environments [12].

Life-history theory provides a framework for investigating the evolution of animal personalities [13,14,15]. Animal personality can have a profound influence on life-history traits like growth, fecundity and survival [15,16,17]. Using evolutionary models, Wolf and co-workers [14] demonstrated that life-history tradeoffs promote the evolution of animal personalities. Individuals varying in exploration behaviour inhabited a low-quality resource habitat for a year at the end of which they could stay for a second year or move to a high-quality resource habitat. Superficial explorers, that evolved high levels of boldness in risky games (= proactive), invested more in current reproduction. Conversely, those that invested more in future reproduction were careful explorers, that evolved low levels of boldness in the same risky games (= reactive). These models therefore predict that individuals with different fitness expectations express different personality traits, here exploratory behaviour. The authors concluded that individuals with high expectations of future fitness, who have much to lose and for whom life is valuable, should be more cautious than individuals with low expectations.

Concurring with model predictions, recent evidence shows that individuals expressing reactive personality traits have a lower basal metabolic rate and therefore lower energetic needs [10,11]. Metabolism of reactive individuals could allow them to survive longer by saving more energy than proactive individuals, especially when foraging involves risk-taking. For instance, a personality implying less risk-taking behaviour and conserving energy would favour survival [16,18]. Thus, long-lived individuals should express a reactive-like personality whereas individuals characterized by a low life expectancy should express a proactive-like personality [14].

The *t* haplotype, also called the "*t* complex", a naturally occurring genetic variant in the house mouse (*Mus domesticus*), provides an appropriate case study to investigate this hypothesis and hence fill the gap of empirical data. The *t* haplotype is a selfish genetic element, consisting of many linked genes, showing drive [19]. Its main known fitness effect is a reduction in litter size in matings between heterozygotes due to a recessive lethal allele [20]. Recently, *t* related effects on life-history have been documented. In a free-living population of house mice, female heterozygotes (+/*t*)

live longer than homozygous wildtype females (+/+), with a 30% viability advantage [21]. No difference in survival was found between +/+ and +/*t* males. Although no information is yet available on whether life expectancy positively correlates with fitness in wild house mice, mean life expectancy has been reported to be 100–150 days [22,23] whereas generation time is about 270 days [24]. This indicates that many mice die before they successfully reproduce, thus suggesting that a higher life expectancy could improve the chance to reproduce.

Following theory on the evolution of life-history and personality [13,14], we hypothesize that reactive personality traits co-evolved with the *t* haplotype. We therefore assessed personality traits in mice of both sexes and genetic backgrounds. We predicted that +/*t* females, characterized by a high survival rate, should express "reactive-like" personality traits and therefore be more shy, less active and less explorative compared to +/+ females, characterized by a lower survival rate. Moreover, we compared the propensity of +/+ and +/*t* to form routine as it has been shown to reflect individuals' ability to use information on their environment and then adapt to its potential changes [7,9,25]. House mice travel their territory daily, covering and marking the same routes repeatedly. Through these routines, mice acquire highly habitual responses, which they can perform rapidly and with minimal sensory input [26]. As proactive individuals form routines faster than reactive individuals, we expect +/+ females to form such routines faster than +/*t* females. Finally, as an index of energy intake we monitored food consumption, expecting that reactive individuals, here +/*t* females, ingest less food compared with proactive individuals, here +/+ females [10,11]. No differences were expected between males of different haplotypes as they have a similar survival rate.

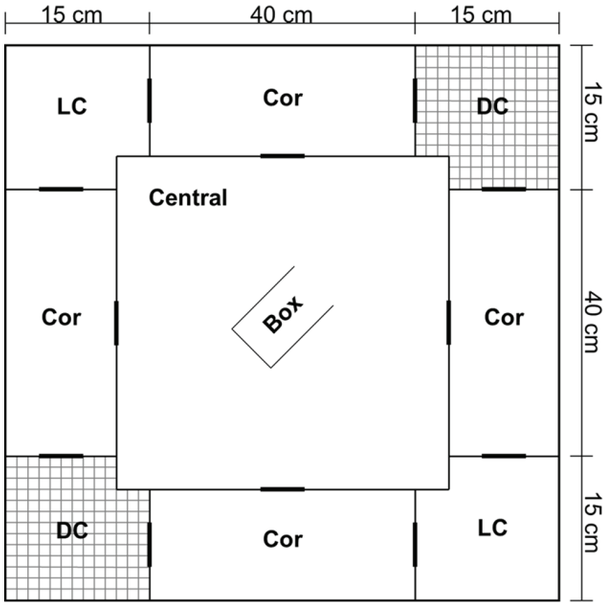

Figure 1. Concentric square field cage to test exploratory behaviour. CC = Covered Corners; Cor = Corridors; UC = Uncovered Corners; Central = Central Compartment; Box = dark box in which mice were transferred to the experimental cage. The holes, drawn in bold in the figure, that connect corridors with corners are 10 cm above the ground whereas the holes that connect the central area with the corridors are 1.5 cm above the ground.
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Methods

Ethics Statement

Animal use and experimental design were approved by the Veterinary Office Zürich, Switzerland (Kantonales Veterinäramt Zürich, no. 97/2009).

Study Subjects

We used 82 sexually mature but non-breeding house mice (more than six weeks old; mean age ± SE = 184±10 days) which were laboratory born F2 and F3 descendants of wild-caught individuals from the same population in the vicinity of Zürich as the one in which longevity differences were reported [21]. We tested a total of 41 females (20 were +/+ and 21 were +/*t*) and 41 males (20 were +/+ and 21 were +/*t*) randomly selected from offspring of our breeding stock. No significant difference in age was observed between +/+ and +/*t* mice of the same sex (females: $t_{39} = 0.03$, $p = 0.973$; males: $t_{39} = 0.84$, $p = 0.408$). Males were younger than females ($t_{80} = 4.02$, $p < 0.001$), because high aggression among males meant that they could not long be housed in groups. All individuals were in good condition for the entire duration of the study.

Housing

All mice were singly housed in Macrolon Type II cages (267×207×140 mm), beginning 5 days before the first behavioural test. Each cage contained standard animal bedding (Lignocel Hygienic Animal Bedding, JRS), an empty toilet paper roll and some paper towel as hides and nest building material. Food (laboratory animal diet for mice, Provimi Kliba SA, Kaiseraugst, Switzerland) and water were provided *ad libitum*. Animals were kept under standardized laboratory conditions at a temperature of 22°C ±3°C with a relative humidity of 50–60% and on a 14:10 light:dark cycle with a 1 h sunrise and dusk phase at the beginning and end of the light phase.

Body Weight

Mice were weighed twice at a 7-day interval with the first measurement the day before the first behavioural test and the second on the day following the end of the first series of behavioural tests. We did not observe significant changes in body weight ($t_{81} = 1.69$, $p = 0.095$). As the two measurements were highly repeatable ($R = 0.95$, $F_{81,82} = 40.52$, $p < 0.001$), we used the mean.

Genotype Determination

An individual ear tissue sample was collected from all males and females at least one week before testing. DNA was isolated and amplified at the *Hba-ps4* locus, a marker containing a 16-bp *t* haplotype specific insertion [27]. PCR products were electrophoresed using an ABI 3730x1 and visualized using Genemapper 4.0 software (Applied Biosystems) to determine genotype at the *t* locus.

Schedule for the Assessment of Personality Traits

For breeding convenience this study was realized in two sessions. The first session took place in February – March whereas the second session took place in July – August. Each behavioural test was performed twice with a seven day interval to check for individual consistency through time [28,29,30]. Exploration tests were however replicated after nine days because of a time constraint. Activity and boldness tests were performed in the morning (from 8:00 to 11:00), whereas the first assessment of exploratory behaviour was performed in the afternoon (15:00 to

Table 1. Individual consistency of the behavioural variables assessed twice at a one-week interval, estimated firstly from mixed model analysis accounting for genetic background, body weight, sex, session, trial and interactions, and secondly from ANOVA-based intra-class correlation coefficients.

Personality traits	Parameters	ID as a random effect		Intra-class correlation coefficients		
		Likelihood ratio	*p*	*R*	$F_{81,82}$	*p*
Activity	number of movements	36.05	<0.0001	0.73	6.49	<0.001
Boldness	number of visits to soiled cat bedding	7.36	0.007	0.31	1.91	0.002
	time spent in soiled cat bedding	0.01	0.999	−0.08	0.85	0.759
Exploration	total number of visits in compartments	23.49	<0.0001	0.43	2.53	<0.001
	time needed to visit all compartments	23.94	<0.0001	0.48	2.82	<0.001

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18:00) and the replicate in the morning. All behaviour tests lasted ten minutes, with the observer standing immobile at a one meter distance. As the activity and boldness tests were performed using the home cage of the mice, the stress induced by the procedures was very limited. Within a three minute acclimation period the mice were very calm and were observed grooming themselves. A single mouse was involved in only one experiment per day and had one day free after each behavioural test. The behavioural tests were run blindly with regard to the genotype of the mice.

Activity

To measure individual activity, we removed nest material and the paper roll from the home cage to facilitate observations. We replaced the cage lid by a Plexiglas lid with a grid drawn on it to uniformly split the cage widthwise into three equal parts. After a three minute acclimation period, the observer recorded the number of times a mouse crossed the lines with all four paws for ten minutes. We then calculated an activity score following previous common procedures [31,32].

Exploration

Exploratory behaviour was assessed in a concentric square field cage representing an arena composed of nine compartments, a central part surrounded by four corridors joined alternatively by covered and uncovered corners [33,34] (Figure 1). After each trial, the apparatus was cleaned with acetone to remove scent marks [35]. A focal mouse was transferred in a small dark box from its home cage to the apparatus to reduce stress before the beginning of the test. The door of the box was aimed at the direction of a covered corner in the first trial and at the direction of an uncovered corner in the replicate. The sliding door of the box was opened by remote control (using a string), and latency time to leave the box, time needed to enter each compartment, and total number of visits to compartments were recorded. For convenience latencies were subtracted from the total duration of the test (600 seconds) such that highly explorative individuals, characterized by short latencies, received a high value.

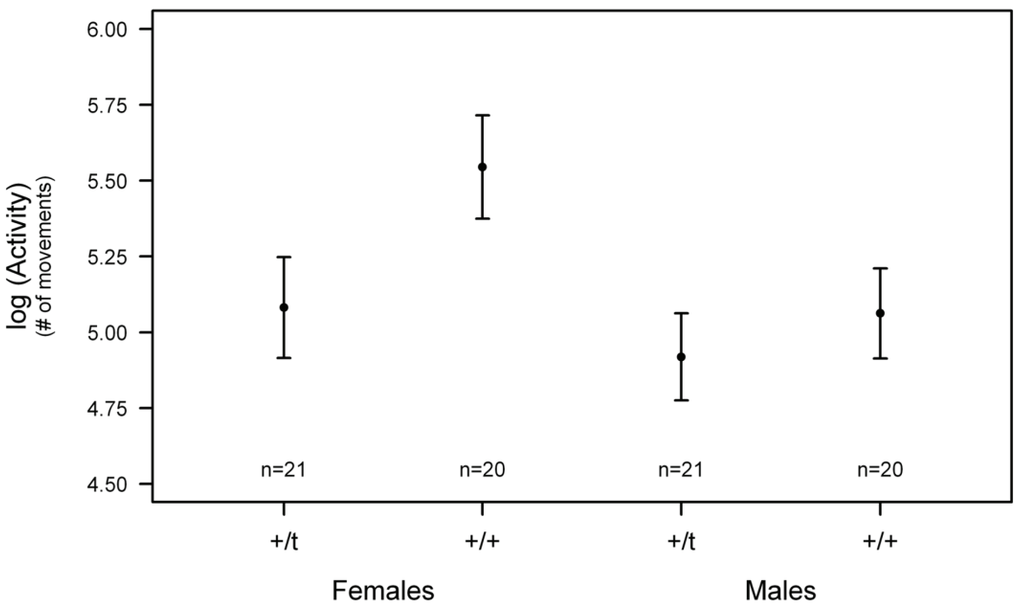

Figure 2. Effect of the genetic background and sex on activity score (mean ± standard errors predicted by mixed effect model).
doi:10.1371/journal.pone.0067130.g002

Table 2. Mixed model analysis of the personality traits showing individual consistency.

	Personality traits							
	Activity		Boldness		Exploration			
	# movements		# visits to soiled bedding		# visits in compartments		time needed to visit compartments	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Genetic background	5.37	0.023	0.03	0.853	1.76	0.188	0.21	0.650
Sex	5.88	0.018	1.91	0.171	1.14	0.289	0.42	0.517
Body weight	2.69	0.105	0.94	0.337	5.58	0.021	0.04	0.847
Session	30.21	<0.001	27.40	<0.001	78.23	<0.001	0.11	0.739
Trial	1.10	0.298	25.31	<0.001	75.77	<0.001	29.68	<0.001
Genetic background : Sex	5.20	0.025	0.09	0.770	1.04	0.312	2.51	0.118
Sex : Trial	0.07	0.787	0.10	0.754	0.02	0.876	0.78	0.380
Genetic background : Trial	0.02	0.877	1.40	0.241	0.66	0.418	0.02	0.881
Genetic background : Sex : Trial	0.18	0.671	1.12	0.293	6.52	0.013	0.29	0.592

doi:10.1371/journal.pone.0067130.t002

Boldness

Boldness was assessed in a classical olfactory test realized with three Macrolon type II cages connected by tubes [36,37]. We connected a central cage to two cages, one at each side. The central cage was filled with bedding from the home cage of the individual tested. The two other cages were filled with either unused cat bedding for one or with soiled cat bedding for the other (Cat's Best Öko Plus, Qualipet). The soiled cat bedding had been used by a domestic cat during one week before the experiment. Cats represent a natural predator against which mice should have evolved avoidance mechanisms [38,39]. Following Dickman & Doncaster [40], mice should be able to assess the presence of predators indirectly through olfactory cues and avoid areas with predator's faeces or urine. Our setting thus represents two identical areas, one of which has apparently been visited by a natural predator, allowing a test of boldness in the face of predator cues [41,42,43]. This procedure avoids a repeated exposure to a real predator, known to be highly stressful for mice [34].

Focal individuals were released in the central cage and kept there for a three minute acclimation period. Removable wire mesh partitions closed the tubes, allowing odour identification of the neighbour cages. At the start of the trial, partitions were removed and the time spent and the number of visits to each cage containing each type of cat bedding were recorded for ten minutes. The mice gave significantly more visits to ($t_{81} = -3.25$, $p = 0.002$) and spent significantly more time ($t_{81} = -2.88$, $p = 0.005$) in the cage filled with unused cat bedding than in the cage filled with soiled cat bedding.

Propensity to Form Routine

Routine formation is usually measured by changing a familiar environment that has been experienced repeatedly and subse-

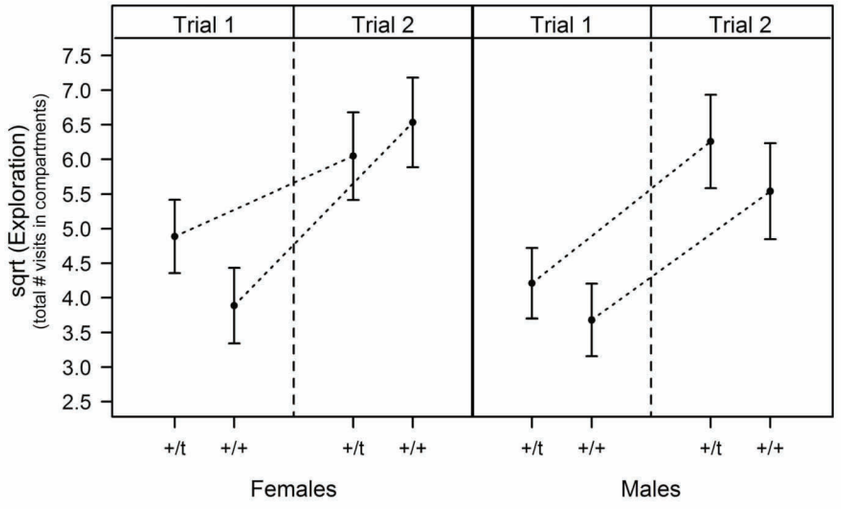

Figure 3. Effect of genetic background and sex on the propensity to form an exploratory routine: increase in exploratory behaviour (total number of visits to all compartments of the exploration apparatus) between the two trials (mean ± standard errors predicted by mixed effect model).
doi:10.1371/journal.pone.0067130.g003

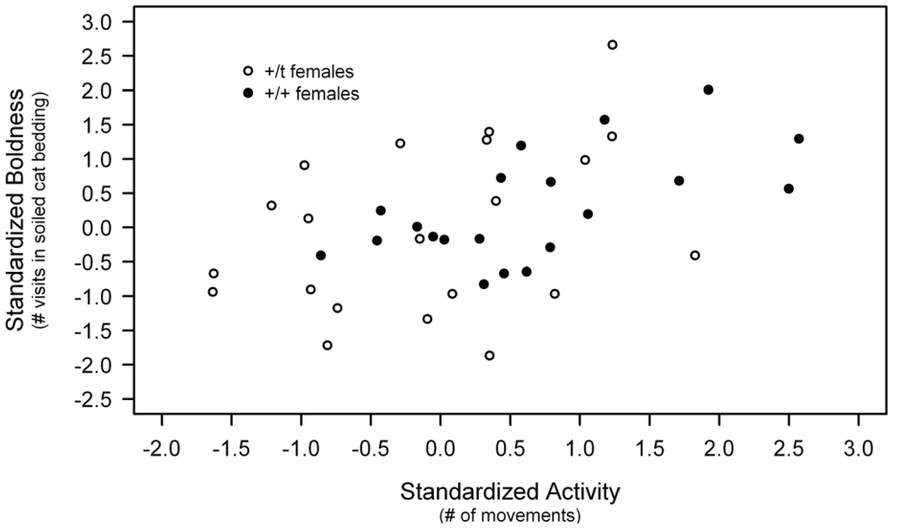

Figure 4. Activity - Boldness syndrome in females, according to genetic background.
doi:10.1371/journal.pone.0067130.g004

quently testing how quickly individuals react to this environmental change [7,9,44]. The propensity to form routine can be indirectly measured by the magnitude of the increase in the performance of a given behaviour between the replicated trials of the same test. Following this idea, we quantified the propensity to form routine as the difference between the performance measured at the second trial and the performance measured at the first trial.

Food Consumption

Food consumption was only recorded for the 48 mice taking part in the second session because of a time constraint at the end of the first session. This sub-sample was composed of 23 females (12+/*t* and 11+/+) and 25 males (9+/*t* and 16+/+). During two consecutive weeks, one month after all behavioural experiments were carried out, the quantity of pellets eaten by the mice was recorded at the same time of day. On day 1 the food holder was cleaned and filled with new pellets of known quantity (weighed on an electronic balance, Sartorius BL 1500 S, with 0.01 g. precision). At day 7 and 14, uneaten pellets were removed for weighing, and at day 7 replaced with new pellets. We checked daily if pieces of pellets had fallen through the feeder grid into the bedding. When found, they were removed and weighed. Food consumption was repeatable between the two weeks (intra-class correlation coefficient: $R = 0.44$, $F_{47,48} = 2.55$, $p < 0.001$).

Statistical Analyses

Statistical tests were carried out using R version 2.13.1 (R development core team 2011). Numbers of visits in the cage containing soiled cat bedding, number of visits in the cage containing clean cat bedding, and total number of visits in all compartments of the exploration apparatus test were square-root transformed, while activity scores, the time needed to explore all the compartments in the exploration test, and quantity of food eaten were log-transformed to satisfy normality.

We tested the influence of individual identity, the genetic background, sex, body weight, session and trial on the variables measured using linear mixed effect models. Interactions between genetic background and sex, trial and sex, trial and genetic background, and between trial, genetic background and sex were also included.

Table 3. Correlations between personality traits showing individual consistency.

				Males						Females					
Behaviour pairs		**All individuals**		**All genotypes**		**+/+**		**+/*t***		**All genotypes**		**+/+**		**+/*t***	
		Pearson r	*p*	*Pearson r*	*p*	*Pearson r*	*p*	*Pearson r*	*p*	*Pearson r*	*p*	*Pearson r*	*p*	*Pearson r*	*p*
Boldness× Activity	# visits cat bedding×# movements	**0.31**	**0.005**	0.06	0.716	0.26	0.259	−0.23	0.312	**0.47**	**0.002**	**0.62**	**0.004**	0.37	0.101
Boldness× Exploration	# visits cat bedding×# visits	0.19	0.080	0.30	0.059	0.45	0.047	0.11	0.643	0.07	0.649	0.16	0.512	0.03	0.882
Activity× Exploration	# movements×# visits	0.16	0.147	0.14	0.383	0.35	0.131	0.05	0.831	0.14	0.396	0.26	0.274	0.07	0.763

Correlations remaining significant after Benjamini & Hochberg correction procedure are in bold.
doi:10.1371/journal.pone.0067130.t003

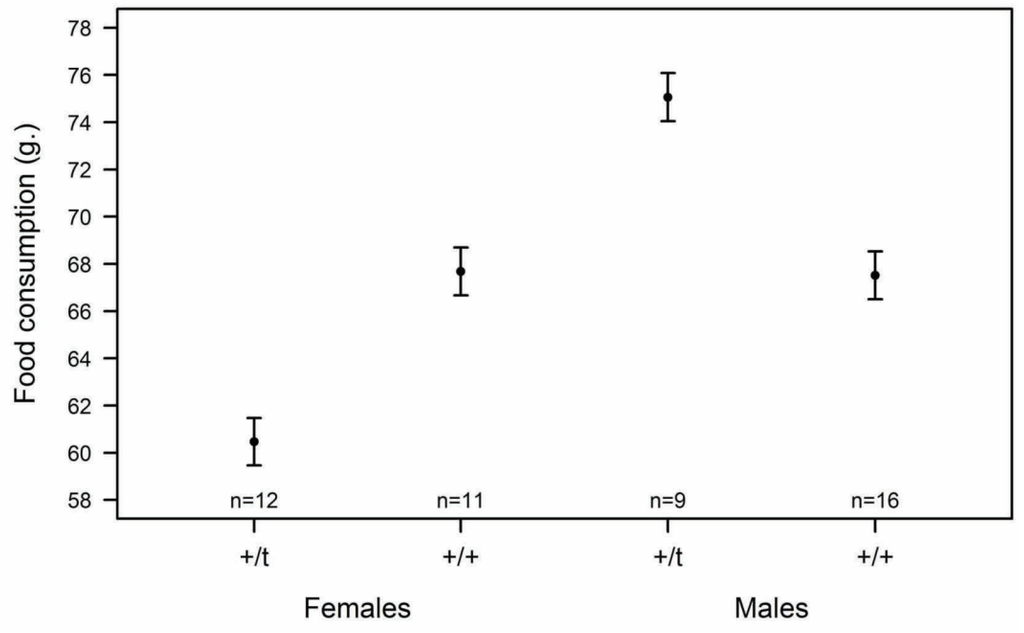

Figure 5. Influence of genetic background and sex on food consumption controlled for body weight (mean ± standard errors predicted by linear regression model).
doi:10.1371/journal.pone.0067130.g005

Individual identity was defined as a random effect to assess individual consistency (repeatability) while all other variables were defined as fixed effects. Significance of the random effect was determined by likelihood ratio tests while fixed effects were tested using F tests [45]. We also used ANOVA-based intra-class correlation coefficients (R) to quantify individual consistency between the two trials of each behavioural test [46,47]. A significant effect of trial in the mixed effect models described above revealed a propensity to form routine. Potential effects of genetic background, sex or their interaction on routine formation were therefore assessed by the effect of the interactions involving trial in the same mixed effect models.

Multiple correlations between the personality traits showing individual consistency enabled us to check for correlations between personality traits. To avoid type I errors, we followed the Benjamini & Hochberg procedure that also reduced type II errors by controlling false discovery rate [48,49]. Beforehand, the number of movements in the activity test, total number of visits to compartments in the exploration test, and the number of visits to cages containing clean and soiled cat bedding were averaged and then standardized (for each session separately) to control for the "session" effect found in the mixed effect models. For each trial the standardized variables are thus defined by an identical mean (equal to 0) and standard deviation (equal to 1).

Food consumption (total food consumed over two weeks) was normally distributed and was analysed using a general linear model to determine the influence of the genetic background, sex, body weight and their interactions. Non significant interactions ($p<0.05$) were dropped from the full model by a backwards stepwise procedure, following Crawley [45].

Results

Individual Consistency

The number of movements during the activity test, the total number of visits to and the time needed to explore all the compartments in the exploration test, and the numbers of visits to the cage containing soiled cat bedding during boldness tests were consistent within an individual through time (Table 1). These variables were therefore used to test for behavioural syndromes.

Personality Traits

The analyses of the influence of the genetic background, sex, and body weight on the personality traits showed that both the t haplotype, sex and their interaction had a significant effect on basic activity (Table 2). $+/t$ females were less active than +/+ females, and females were in general more active than males (Figure 2). None of the personality traits measured in the boldness and exploration tests were influenced by the genetic background, sex or their interaction (Table 2). Body weight did not have any significant effect in any of the personality traits except for the total number of visits in the exploration test (Table 2).

Propensity to Form Routine

No propensity to form routine was observed in the activity test as mice showed similar activity scores between the first and the second trial (Table 2). However, during the boldness test the number of visits increased during the second trial to both the cage with soiled cat bedding (1^{st} trial (mean ± SE): 5.5±0.6, 2^{nd} trial: 8.4±0.6) and the cage with clean cat bedding (1^{st} trial (mean ± SE): 6.6±0.6, 2^{nd} trial: 9.0±0.6) (Table 2). During the exploration test, the total number of visits to the compartments increased between the first and the second trial (1^{st} trial: 23.1±2.7, 2^{nd} trial: 45.9±4.8) whereas the time needed to explore all the compartments decreased (1^{st} trial: 560±12 sec., 2^{nd} trial: 468±22 sec.),

both suggesting a propensity to form an exploratory routine (Table 2).

Genetic background, sex or their interaction did not have any significant influence on the propensity to form a routine observed in the boldness test, as measured by the number of visits to the cage containing soiled cat bedding or the number of visits to the cage containing clean cat bedding (Table 2). The analysis of the propensity to form an exploratory routine as measured by the increase in the total number of visits in the exploration test did not show an overall influence of sex or genetic background but a significant effect of the interaction of genetic background with sex (Table 2). Heterozygous +/*t* females were less prone to form an exploratory routine than +/+ females as they had a lower increase in their number of visits whereas there was no significant difference between +/*t* and +/+ males (Figure 3). When analysing the decrease in the time needed to visit all the compartments between the two replicates, sex, genetic background or their interaction did not show any significant effect on the formation of an exploratory routine (Table 2).

Correlations between Personality Traits

The positive relationship between boldness and activity allowed us to define a behavioural syndrome in females but not in males (Table 3). More precisely, this relationship was significant in +/+ females whereas +/*t* females only showed a non-significant tendency to express it (Figure 4; Table 3).

Food Consumption

Even though genetic background ($F_{1,46} = 0.12$, $p = 0.73$), sex ($F_{1,45} = 3.05$, $p = 0.09$), and body weight ($F_{1,44} = 2.56$, $p = 0.12$) did not show an overall influence on food consumption, the interaction between genetic background and sex had a marginally significant effect ($F_{1,43} = 3.72$, $p = 0.06$). Whereas +/+ males ate less than +/*t* males (+/+ males: 69.01±3.79 g., +/*t* males: 76.95±6.23 g.), the opposite was true in females, as +/*t* females ate less than +/+ females (+/*t* females: 61.02±2.59 g., +/+ females: 68.27±2.88 g.; Figure 5).

Discussion

Our study demonstrated that laboratory reared female house mice of a genotype conferring a survival advantage under natural conditions expressed reactive-like behavioural traits favouring cautiousness and energy conservation. The longer living +/*t* females were less active, less prone to form an exploratory routine, and tended to ingest less food than the shorter living +/+ females.

Having a low activity level could have various positive effects on survival. First, decreasing activity can be beneficial for small rodents when facing predators relying on hearing or sight to detect prey [50]. Second, organismal maintenance requires partitioning of the available energy budget to different biological functions among which effector organs like skeletal muscles are responsible for much of the daily energy expenditure [51]. Within a given energy budget, an individual with a reduced activity can attribute a large part of its energy budget to other functions that could improve survival.

Our results on food consumption supported our energy-saving interpretation as +/*t* females showed a tendency to have a lower food intake than +/+ females. This could reflect a lower need for energy and/or a better capacity to save energy that could both favour survival when access to food is restricted or risky. Our results suggest that reactive individuals could decrease the frequency of their visits to feeding places compared to proactive individuals and may decrease the risk of being caught by predators when feeding. Moreover, research on rate of aging in rodents showed that mice fed with a 65% reduced diet improve their maximum life span by 51% compared to mice fed *ad libitum* [52]. Caloric restriction extends life span through mechanisms such as reduced oxidative damage [53]. This could also apply to +/*t* females and hence would partly explain their survival advantage over +/+ females that have a higher food consumption.

Moreover, +/*t* females were less prone to form an exploratory routine. Although reactive and proactive individuals have similar learning abilities, at least in birds, reactive individuals form routines slower than proactive individuals [9,25]. This particularity, seen as a higher attentiveness to the environment, confers an advantage to reactive individuals as they can better adjust to sudden environmental changes than proactive individuals [7,54,55].

Conversely to other personality studies, we did not observe behavioural syndromes between most of the personality traits we assessed [5,6]. We found a syndrome defined by a positive correlation between activity and boldness, such that the less active females were also the more cautious. However, this relationship was significant in +/+ females whereas +/*t* females only showed a tendency. Some studies have shown that behavioural syndromes are not ubiquitous, even within the same species. In three-spined sticklebacks (*Gasterosteus aculeatus*) the presence of behavioural syndromes depends on whether population characteristics favour suites of correlated behaviours [32,56,57]. The absence of behavioural syndromes in male house mice could thus be due to sex-specific behavioural optima.

The differences observed in the activity test are consistent with expected differences in energy demands due to milk production. Costs of lactation are very high in small rodents and increase with litter size [58]. Litter size is influenced by the *t* haplotype. On average +/*t* females have smaller litters than +/+ females, as whenever +/*t* females mate with +/*t* males their litter sizes are nearly halved due to the lethal homozygous effect of the *t* haplotype [59]. Thus a female's expected average litter size should correlate with activity levels. Higher activity levels help to gather information about food to cover energetic needs during lactation. Consistent with this, we showed for non-breeding mice that +/*t* females had lower activity levels than did +/+ females. Fitness of +/*t* and +/+ females will on average be equal if +/*t* females compensate for smaller litters by producing more litters, which greater longevity would permit. This would contribute to maintaining the polymorphism in the population. Perrigo [60] showed that lactation strongly influences activity patterns of females, and that males were less active than females. We also found that males were less active than females.

The lack of difference in exploration and boldness between mice of different sexes and genotype suggests that balancing selection has resulted in a single optimal behavioural level for each, with no correlation between individual values for each traits. House mice in western Europe live commensally with humans and nearly always are found close to easily accessible food resources, and often in dense population [26,38], suggesting that exploration to find new food patches may often be secondary to exploration to monitor social situations. Both males and females monitor the presence of conspecifics and defend their territories against intruders [61]. Similarly, boldness behaviour might be under strong balancing selection pressure reducing inter-individual variability, the raw material needed to evolve personalities.

Although our study provides interesting insights into personality traits associated with +/*t* females and survival differences, the causal relationship is unclear. The *t* haplotype, consisting of a third of chromosome 17, has had an independent evolutionary history

from its wildtype counterpart for more than two million years [62]. Major Histocompatibility Complex genes are located within the four inversions comprising the *t* haplotype [63] and there is evidence that a gene influencing both male and female mate choice is also located within the *t* haplotype [20]. Genes influencing other traits, such as personality and/or survival, either additively or epistatically or through dominance, could be located within this region.

Behavioural studies like ours do not only help in understanding the *t* haplotype but also underline new questions related to life-history trade-offs and the evolution of animal personalities [13,14,64]. The rate-of-living theory postulates a negative association between life span and the rate of energy expenditure [65]. Thus two opposite strategies "live fast and die young" or "live slowly and die old", define a fast-slow life-history continuum along which individuals can be ranked [66,67,68]. Our results give evidence that these two life-history strategies apply to the *t* complex, with +/+ females living extravagantly and +/*t* females living frugally. However, Wolf et al. [14] predicted an association between residual reproductive value and risk-related behaviours like exploration or boldness so that we could expect +/*t* females to be shyer and less explorative than +/+ females. However, our results fail to provide full empirical support to theory as only activity showed a clear association with the *t* haplotype and we did not find a strong relationship between activity and boldness.

Literature provides few examples reporting the influence of personality traits like activity, aggressiveness, and sociality on reproductive success or longevity [69,70,71] (see [72] for a review). Our study indicates that longer living house mice express reactive personality traits, demonstrating that longevity correlates with personality. However, as studies focusing on life-history productivity and personality are still missing in this species, we do not know if the expression of specific personality traits could also influence their reproductive success and/or tactics [13,64].

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Author Contributions

Conceived and designed the experiments: YA. Performed the experiments: YA. Analyzed the data: YA. Wrote the paper: YA BK AKL.

References

1. Réale D, Dingemanse NJ, Kazem AJN, Wright J (2010) Evolutionary and ecological approaches to the study of personality. Phil Trans R Soc B 365: 3937–3946.
2. Réale D, Reader SM, Sol D, Mcdougall PT, Dingemanse NJ (2007) Integrating animal temperament within ecology and evolution. Biol Rev 82: 1–28.
3. David M, Auclair Y, Cézilly F (2012) Assessing short- and long-term repeatability and stability of personality in captive zebra finches using longitudinal data. Ethology 118: 932–942.
4. Bell AM (2007) Future directions in behavioural syndromes research. Proc R Soc B 274: 755–761.
5. Wilson ADM, Whattam EM, Bennett R, Visanuvimol L, Lauzon C, et al. (2010) Behavioral correlations across activity, mating, exploration, aggression, and antipredator contexts in the European house cricket, *Acheta domesticus*. Behav Ecol Sociobiol 64: 703–715.
6. David M, Auclair Y, Cézilly F (2011) Personality predicts social dominance in female zebra finches, *Taeniopygia guttata*, in a feeding context. Anim Behav 81: 219–224.
7. Benus RF, Den Daas S, Koolhaas JM, van Oortmerssen GA (1990) Routine formation and flexibility in social and non-social behaviour of aggressive and non-aggressive male mice. Behaviour 112: 176–193.
8. Koolhaas JM, Korte SM, De Boer SF, van Der Vegt BJ, van Reenen CG, et al. (1999) Coping styles in animals: current status in behavior and stress-physiology. Neurosci Biobehav Rev 23: 925–935.
9. Marchetti C, Drent PJ (2000) Individual differences in the use of social information in foraging by captive great tits. Anim Behav 60: 131–140.
10. Careau V, Thomas D, Humphries MM, Réale D (2008) Energy metabolism and animal personality. Oikos: 641–653.
11. Careau V, Bininda-Emonds ORP, Thomas DW, Réale D, Humphries MM (2009) Exploration strategies map along fast-slow metabolic and life-history continua in muroid rodents. Funct Ecol 23: 150–156.
12. Dall SRX, Houston AI, McNamara JM (2004) The behavioural ecology of personality: consistent individual differences from an adaptive perspective. Ecol Lett 7: 734–739.
13. Biro PA, Stamps JA (2008) Are animals personality traits linked to life-history productivity? Trends Ecol Evol 23: 361–368.
14. Wolf M, Sander van Doorn G, Leimar O, Weissing FJ (2007) Life-history trade-offs favour the evolution of animal personalities. Nature 447: 581–584.
15. Stamps JA (2007) Growth-mortality tradeoffs and "personality traits" in animals. Ecol Lett 10: 355–363.
16. Boon AK, Réale D, Boutin S (2008) Personality, habitat use, and their consequences for survival in North American red squirrels *Tamiasciurus hudsonicus*. Oikos 117: 1321–1328.
17. Both C, Dingemanse NJ, Drent PJ, Tinbergen JM (2005) Pairs of extreme avian personalities have highest reproductive success. J Anim Ecol 74: 667–674.
18. Clark CW (1994) Antipredator behavior and the asset-protection hypothesis. Behav Ecol 5: 159–170.
19. Lyon MF (2003) Transmission ratio distortion in mice. Annu Rev Genet 37: 393–408.
20. Lenington S (1991) The *t* complex: a story of genes, behavior, and population. Adv Study Behav 20: 51–86.
21. Manser A, Lindholm A, König B, Bagheri H (2011) Polyandry and the decrease of a selfish genetic element in a wild house mouse population. Evolution 65: 2435–2447.
22. Pennycuik PR, Johnston PG, Westwood NH, Reisner AH (1986) Variation in numbers in a house mouse population housed in a large outdoor enclosure: seasonal fluctuations. J Anim Ecol 55: 371–391.
23. Berry RJ, Jakobson ME (1971) Life and death in an island population of the house mouse. Exp Geront 6: 187–197.
24. Manser A (2009) The influence of a selfish genetic element (*t* haplotype) on a wild house mouse population. Zürich: University of Zürich.
25. Guillette LM, Reddon AR, Hurd PL, Sturdy CB (2009) Exploration of a novel space is associated with individual differences in learning speed in black-capped chickadees, *Poecile atricapillus*. Behav Process 82: 265–270.
26. Latham N, Mason G (2004) From house mouse to mouse house: the behavioural biology of free-living *Mus musculus* and its implications in the laboratory. Appl Anim Behav Sci 86: 261–289.
27. Hammer MF, Schimenti J, Silver LM (1989) Evolution of mouse chromosome 17 and the origin of inversions associated with *t* haplotypes. Proc Natl Acad Sci USA 86: 3261–3265.
28. Réale D, Gallant BY, Leblanc M, Fiesta-Bianchet M (2000) Consistency of temperament in bighorn ewes and correlates with behaviour and life history. Anim Behav 60: 589–597.
29. van Oers K, Drent PJ, De Goede P, van Noordwijk AJ (2004) Realized heritability and repeatability of risk-taking behaviour in relation to avian personalities. Proc R Soc B 271: 65–73.
30. Carere C, Drent PJ, Privitera L (2005) Personalities in great tits, *Parus major*: stability and consistency. Anim Behav 70: 795–805.
31. Quinn JL, Cresswell W (2005) Personality, anti-predation behaviour and behavioural plasticity in the chaffinch *Fringilla coelebs*. Behaviour 142: 1377–1402.
32. Bell AM (2005) Behavioural differences between individuals and two populations of stickleback (*Gasteroteus aculeatus*). J Evol Biol 18: 464–473.
33. Augustsson H, Meyerson BJ (2004) Exploration and risk assessment: a comparative study of male house mice (*Mus musculus musculus*) and two laboratory strains. Physiol Behav 81: 685–698.
34. Marques JM, Olsson IAS, Ögren SO, Dahlborn K (2008) Evaluation of exploration and risk assessment in pre-weaning mice using the novel cage test. Physiol Behav 93: 139–147.
35. Hurst JL (1989) The complex network of olfactory communication in populations of house mice *Mus domesticus* Rutty: urine marking and investigation within family group. Anim Behav 37: 705–725.
36. Lenington S, Egid K, Williams J (1988) Analysis of a genetic recognition system. Behav Gen 18: 549–564.
37. Nunes AC, Maria da Luz M, Ganem G (2009) Odor preferenece in house mice: influences of habitat heterogeneity and chromosomal incompatibility. Behav Ecol 20: 1252–1261.

38. Berry RJ (1970) The natural history of the house mouse. Field Stud 3: 219–262.
39. Dickman CR (1992) Predation and habitat shift in the house mouse, *Mus domesticus*. Ecology 73: 313–322.
40. Dickman CR, Doncaster CP (1984) Responses of small mammals to red fox (*Vulpes vulpes*) odour. J Zool Lond 204: 521–531.
41. Lima SL, Dill LM (1990) Behavioral decisions made under the risk of predation: a review and prospectus. Can J Zool 68: 619–640.
42. Pillay N, Alexander GJ, Lazenby SL (2003) Responses of striped mice, Rhabdomys pumilio, to faeces of a predatory snake. Behaviour 140: 125–135.
43. Jedrzejewski W, Rychlik L, Jedrzejewska B (1993) Responses of bank voles to odours of seven species of predators: experimental data and their relevance to natural predator-vole relationships. Oikos 68: 251–257.
44. Benus RF, Koolhaas JM, van Oortmerssen GA (1987) Individual differences in behavioural reaction to a changing environment in mice and rats. Behaviour 100: 105–122.
45. Crawley MJ (2007) The R book. Chichester, England: John Wiley & Sons Ltd.
46. Nakagawa S, Schielzeth H (2010) Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. Biol Rev 85: 935–936.
47. Lessells CM, Boag PT (1987) Unrepeatable repeatabilities: a common mistake. Auk 104: 116–121.
48. Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc 57: 289–300.
49. Verhoeven KJF, Simonsen KL, McIntyre S, McIntyre L (2005) Implementing false discovery rate control: increasing your power. Oikos 108: 643–647.
50. Apfelbach R, Blanchard CD, Blanchard RJ, Hayes RA, McGregor IS (2005) The effects of predator odors in mammalian prey species: a review of field and laboratory studies. Neurosci Biobehav Rev 29: 1123–1144.
51. Ricklefs RE, Konarzewski M, Daan S (1996) The relationship between basal metabolic rate and daily energy expenditure in birds and mammals. Am Nat 147: 1047–1071.
52. Weindruch R, Walford RL, Fligiel S, Guthrie D (1986) The retardation of aging in mice by dietary restriction: longevity, cancer, immunity and lifetime energy intake. J Nutr 116: 641–654.
53. Mattson MP (2005) Energy intake, meal frequency, and health: a neurobiological perspective. Annu Rev Nutr 25: 237–260.
54. Exnerová A, Svádová KH, Fucíková E, Drent PJ, Stys P (2010) Personality matters: individual variation in reactions of naive bird predators to aposematic prey. Proc R Soc B 277: 723–728.
55. Guillette LM, Reddon AR, Hoeschele M, Sturdy CB (2011) Sometimes slower is better: slow-exploring birds are more sensitive to changes in a vocal discrimination task. Proc R Soc B 278: 767–773.
56. Bell AM, Sih A (2007) Exposure to predation generates personality in threespined sticklebacks (*Gasterosteus aculeatus*). Biol Lett 10: 828–834.
57. Dingemanse NJ, Wright J, Kazem AJN, Thomas DK, Hickling R, et al. (2007) Behavioural syndromes differ predictably between 12 populations of three-spined stickleback. J Anim Ecol 76: 1128–1138.
58. König B, Riester J, Markl H (1988) Maternal care in house mice (*Mus musculus*): II. The energy cost of lactation as a function of litter size. J Zool Lond 216: 195–210.
59. Lindholm AK, Musolf K, Weidt A, König B (2013) Mate choice for genetic compatibility in the house mouse. Ecol Evol 3: 1231–1247.
60. Perrigo G (1990) Food, sex, time, and effort in a small mammal: energy allocation strategies for survival and reproduction. Behaviour 114: 191–205.
61. Gray SJ, Plesner Jensen S, Hurst JL (2000) Structural complexity of territories: preference, use of space and defence in commensal house mice, *Mus domesticus*. Anim Behav 60: 765–772.
62. Morita T, Kubota H, Murata K, Nozaki M, Delarbre C, et al. (1992) Evolution of the mouse *t* haplotype: recent and worldwide introgression to *Mus musculus*. Proc Natl Acad Sci USA 89: 6851–6855.
63. Hammerberg C, Klein J (1975) Linkage disequilibrium between *H-2* and *t* complexes in chromosome 17 of the mouse. Nature 258: 296–299.
64. Réale D, Garant D, Humphries MM, Bergeron P, Careau V, et al. (2010) Personality and the emergence of the pace-of-life syndrome concept at the population level. Phil Trans R Soc B 365: 4051–4063.
65. Speakman JR, Selman C, McLaren JS, Harper EJ (2002) Living fast, dying when? The link between aging and energetics. J Nutr 132: 1583S–1597S.
66. Gaillard J-M, Pontier D, Allainé D, Lebreton JD, Trouvilliez J, et al. (1989) An analysis of demographic tactics in birds and mammals. Oikos 56: 59–76.
67. Kraus C, Thomson DL, Künkele J, Trillmich F (2005) Living slow and dying young? Life-history strategy and age-specific survival rates in a precocial small mammal. J Anim Ecol 74: 171–180.
68. Bielby J, Mace GM, Bininda-Emonds ORP, Cardillo M, Gittleman JL, et al. (2007) The fast-slow continuum in mammalian life history: an empirical reevaluation. Am Nat 169: 748–757.
69. Boon AK, Réale D, Boutin S (2007) The interaction between personality, offspring fitness and food abundance in North American red squirrels. Ecol Lett 10: 1094–1104.
70. Cote J, Dreiss A, Clobert J (2008) Social personality trait and fitness. Proc R Soc B 275: 2851–2858.
71. Réale D, Martin J, Coltman DW, Poissant J, Festa-Bianchet M (2009) Male personality, life-history strategies and reproductive success in a promiscuous mammal. J Evol Biol 22: 1599–1607.
72. Smith BR, Blumstein DT (2008) Fitness consequences of personality: a meta-analysis. Behav Ecol 19: 448–455.

Chapter 13

The nasty neighbour in the striped mouse (*Rhabdomys pumilio*) steals paternity and elicits aggression

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The nasty neighbour in the striped mouse (*Rhabdomys pumilio*) steals paternity and elicits aggression

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Abstract

Background: Territoriality functions to monopolize access to resources including mates, but is costly in terms of energy and time investment. Some species reduce these costs by being less aggressive towards their neighbours than towards unfamiliar strangers, the so called dear enemy phenomenon. However, in other species individuals are more, not less aggressive towards their neighbours. It has been hypothesised that this is due to the fact that neighbours can impose a greater threat than strangers, but this has not been tested previously.

Results: We tested aggression in wild group-living male striped mice in a neutral test arena and demonstrate that breeders are more aggressive than non-breeding philopatrics, and that more aggression occurs during the breeding than during the non-breeding season. Male breeders were significantly more aggressive towards their neighbours than towards strangers, leading to the prediction that neighbours are the most important competitors for paternity. Using a molecular parentage analysis we show that 28% of offspring are sired by neighbouring males and only 7% by strangers.

Conclusions: We conclude that in male striped mice the main function of male aggression is defending paternity against their territorial neighbours.

Background

Territoriality functions to monopolize resources including food, shelter and access to mates, and is thus a strategy to increase fitness [1,2]. The importance of territoriality in obtaining reproductive success has been demonstrated for example in coyotes (*Canis latrans*) where reproductive success within a population was obtained exclusively by territorial individuals [3]. While territoriality can have significant benefits, it is also costly [4], especially in forms of energy expenditure [5], time requirements and the increased risk of injury [6] and predation [1].

As territoriality is costly, it is not surprising that individuals seek strategies to reduce these costs. One possibility is to reduce territorial aggression towards individuals that are less likely to pose a threat. For example in many species males show less or no aggression towards strange females compared to males [7-13], as females represent potential mates rather than competitors [14]. Another example is the "dear enemy" phenomenon, a case of context-specific territorial response, where territory holders are less aggressive towards familiar neighbours than towards strangers [15,16]. It is believed that floating (= unfamiliar) males pose a greater threat as they seek to obtain a territory, while neighbouring territorial males accept each other's territory boundaries [16].

In contrast to the dear enemy phenomenon is the nasty neighbour phenomenon [17]: in some species, individuals are more aggressive towards their neighbours than towards strangers. It has been suggested that this is the case when neighbours represent a greater threat to territory holders than to strangers [15], but what exactly represents that increased threat is seldom known [17,18]. In red-winged blackbirds (*Agelaius phoeniceus*), which show the dear enemy and not the nasty neighbour phenomenon, it has been shown that males are more aggressive towards sexually attractive neighbours [19]. However, up to date direct evidence that the nasty neighbour phenom-

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enon is due to stronger competition for paternity by neighbours is missing.

In the current field study we measured male aggression in neutral test arenas in the striped mouse (*Rhabdomys pumilio*). The striped mouse is group living with one single breeding male and up to 4 communally breeding females per group [20]. Groups typically contain several philopatric adult sons (and daughters) that are believed not to breed in their natal group [21] and all group members participate in territorial defence [22]. We predicted that male aggression is related to defending paternity in harems, i.e. that breeding males would be more aggressive than natally philopatric males and that male aggression is more pronounced during the breeding than during the non-breeding season. Then we tested for the dear enemy phenomenon by staging encounters between specific individuals. We found that breeders were more aggressive towards their neighbours, which would be in agreement with the nasty neighbour hypothesis. We then predicted neighbours to be the most severe competitors for paternity and tested this using molecular markers to determine paternity of 119 pups born in 9 different social groups, demonstrating extra-group paternity to be high and mainly due to neighbours. Finally, we demonstrated that female choice plays a role in the loss of paternity by the breeding male.

Materials and methods

Study area and period

The study was conducted 2004 to 2007 in Goegap Nature Reserve in South Africa (S 29 41.56, E 18 1.60). The area is arid, with an average rainfall of 160 mm p.a., and the vegetation type is classified as Succulent Karoo. The study received clearance from the animal's ethics committee of the University of the Witwatersrand (2004/87/2A and 2005/82/4).

Study species

Striped mice are diurnal, inhabit an open habitat and are readily habituated to the presence of observers, which allows direct behavioural observations in the field [23]. The breeding season of 3-4 months occurs in spring from August to November (2-3 litters per female, [24]. Males follow one of three tactics [21]: (i) group-living territorial breeding males. These are the breeding males of extended family groups with up to four communally breeding females and several adult philopatric males and females. Groups can contain up to 30 adult individuals of both sexes but only one breeding male [20]. (ii) Group-living philopatric males that stay in their natal group after reaching adulthood. They might seek copulations with non-related females from neighbouring groups (as has been described for other species; [25,26]. (iii) Solitary roamers that try to breed with females of communal groups which are defended by breeding males. Roamers have much larger home ranges than other males, and their home ranges overlap the home ranges of several females [21,27]. Roamers might first go through a phase of floating, when they leave their natal group and try to find a home range. In contrast to males, females are typically philopatric and do not disperse [20].

Trapping, observation and radio-tracking

We studied between 9 and 20 focal groups from 2004 to 2007, with a similar number of non-focal neighbouring groups. Mice from focal groups were trapped, marked, observed and radio-tracked to determine social tactics of males, as described in detail elsewhere [20,21,23], while mice from neighbouring groups were only trapped and marked. The tail tip was taken as tissue sample from each individual for genetic analysis and stored in 90% ethanol.

Measuring aggression

To measure aggressive behaviour, trapped males were tested in a neutral presentation arena (100 × 80 × 65 cm) made of white veneered chipboard. The bottom was lined with plastic foil on which a 2-3 cm layer of sand was provided. The sand was obtained from the dry riverbed going through the field site. To avoid influence from olfactory cues from previous experiments, e.g. faeces or urine, the sand in the arena was changed between experiments and the arena was cleaned with 90% alcohol (for a similar procedure see [28].

Mice were tested in pairs and for each experiment each male was used only once as stimulus animal. A partition (79 × 47 × 1.5 cm chipboard) in the middle of the arena separated the two males for a habituation phase of 5 min. Seven sunflower seeds were given to each male during the 5 min habituation phase to calm down captured wild mice. All mice ate all of their sunflower seeds. Then the partition was removed and the striped mice were observed for 15 min. If the males showed severe aggression (biting), the experiment was immediately terminated before the 15 minutes had elapsed. We recorded all aggressive behaviours (see [22]: chasing, fighting (standing on their hind legs and kicking each other with their forelegs), and biting. Biting was always preceded by either chasing or fighting. We then calculated the frequency of aggressive interactions initiated by the focal male per minute. The aggressive behaviour patterns observed were the same as observed during field experiments and in a similar intensity and frequency [22].

Altogether we tested 27 dyads of breeding males during the breeding season (September and October 2004 and October 2005) and 16 during the non-breeding season in March 2006 and March 2007. 14 breeding males were tested twice during the breeding season (October 2004 and 2005), once with a breeding male neighbouring them,

and the other time with a breeding male not neighbouring their territory. Six males were first tested with their neighbour, the other eight males firsts with the stranger. The average time between experiments a focal male was tested either with the neighbour or the stranger was 6.1 ± 5.1 days. Additionally, we tested 12 dyads of philopatric males during the breeding season 2005 with philopatric males unknown to them (not neighbours).

Paternity analysis

We isolated DNA from mouse tissue using magnetic particle purification (BioSprint 96 DNA Blood Kit, Qiagen). We used 9 polymorphic microsatellite loci from the house mouse genome (Chr13_1, Chr1_12, Chr1_21, Chr2_3, Chr7_64, D3Mit211, Chr11_81, Chr19_18, Chr5_38), primarily from [29], and amplified them for all individuals in two multiplexes using the Qiagen PCR-Multiplex-Kit with a final concentration of 0.1/0.2 μM primer for 35 cycles at an annealing temperature of 60°C). Mean number of alleles per locus was 13.9 (range 10 - 18). Typing error rates for the nine loci were estimated as 0.014 and were strongly influenced by poor repeatability of amplification of one locus in one 96-well plate; if this one plate were excluded from the average, the average error rate then fell below 0.01. The proportion of loci typed was 0.97.

Parentage analyses were performed using Cervus 3.0. Parameters for the simulation of parentage analysis were set as 100,000 offspring, 95% sampling of candidate mothers, 85% sampling of candidate fathers, 0.015 proportion of loci mistyped (to be conservative), and the confidence level was set at 95%. We accepted parentage assignment when trio confidence was 95% and there was zero or one mismatch between each parent and offspring, and no more than two mismatches in the trio of candidate parents and offspring. If trio confidence was less than 95% but a parent-offspring pair met the 95% confidence threshold with one or fewer mismatches, we accepted the maternity or paternity. If both a mother and father of the same offspring could be separately assigned with 95% confidence and one or fewer mismatches, but the trio had a confidence value of less than 95% and/or had more than two mismatches, we awarded parentage to the putative father if its pair delta value with the offspring exceeded that of the putative mother, and *vice versa*.

Altogether, we analysed 119 pups born between 1st September and 1st December 2005 from 9 different social groups. All breeding females and breeding males from these groups were considered as potential parents, as well as all roaming males and breeding males from neighbouring non focal groups (total of 72 males). Tenure of breeding and roaming males at the field site was 2.1 ± 1.0 months. No neighbouring male took over a breeding position at any group during the study period. The breeding male which was present 3 weeks before birth of pups was regarded as a potential father, as parturition is approximately 3 weeks [24]. Additionally, 37 philopatric males of focal groups born at the start of the breeding season were considered as potential fathers for offspring born later in the breeding season.

We excluded 4 offspring from all further analysis, because their mothers were statistically unknown (all candidate mothers had negative loglikelihood scores) and group association of pups could not be confirmed by observations. Of the remaining 115 offspring, success in paternity assignment was 80.8%. We further excluded 14 offspring from statistical analysis whose father was most likely the breeding male of the groups or a neighbouring male (positive LOD score), but did not meet the confidence threshold. Thus, these offspring could not be confirmed as having been sired by an unknown male.

Data analysis

Data are presented as mean ± SE. Data from aggression tests were analysed using non-parametric statistics, because sample sizes were small. The Mann-Whitney U-Test (U) was used for unpaired data, the Wilcoxon matched-pairs signed-ranks test for paired data (T). We used Fisher's Exact test for comparisons of ratios. We estimated multiple paternity and paternity share using maximum likelihood and estimated their confidence intervals by bootstrapping 100,000 times, using the method of Eccard and Wolf [30] in R 2.9.1. Multiple paternity estimates the proportion of litters sired by more than one male. However, larger litters are more likely to show multiple paternities than smaller litters [30]. We therefore also used a second measure, paternity share, that is independent of litter size [30]. Paternity share is an estimate of the probability that an offspring is sired by a male other than the primary male. The median litter size of 3 was used as input, together with the empirically based estimates of multiple paternity in litters (see Results).

Results

Is male aggression related to reproduction?

During the breeding season, breeding males showed 2.0 ± 0.7 aggressive interactions/min, while philopatric males did not show any aggression (0.0 ± 0.0 aggressive interactions/min). During the breeding season, 16 of 27 trials had to be terminated due to high aggression, and 3 of 16 during the non-breeding season ($p < 0.02$, Fisher's Exact test). As the standard deviation for philopatric males was zero, we performed a Fisher's Exact test: 21 of 27 breeding males showed aggression but none of the 12 philopatric males ($p = 0.0002$). Breeding males showed

significantly more aggressive interactions during the breeding (2.0 ± 0.7 aggressive interactions/min, N = 27) than during the non-breeding season (0.2 ± 0.1 aggressive interactions/min, N = 16; p = 0.03, U = 130.50).

Dear or nasty neighbour?

Breeding males tested during the breeding season showed significantly more aggression towards their neighbours (5.0 ± 3.1 aggressive interactions/min) than towards strange breeding males not neighbouring them (1.1 ± 0.4 aggressive interactions/min; p = 0.001, T = 0; paired N = 14; Fig. 1). Nine trials with neighbours and 6 trials with strangers had to be terminated (p > 0.4, Fisher's Exact test)., and the total duration of the experiments until termination (maximum 900 seconds) was shorter with neighbouring males than with strangers (412 ± 404 seconds *versus* 611 ± 380 seconds; p < 0.02, T = 3).

Multiple paternities and extra-group paternity

Of 24 litters, 15 (62.5%) had only one father, 8 had two fathers (33.3%) and one had three fathers (4.2%). Multiple paternity of litters was estimated as 36.0% (95% confidence interval: 16.0% - 56.0%). An alternative estimate of multiple paternity, the paternity share [30], was estimated as 13.9% (95% confidence interval: 5.6% - 24.8%).

Of the 101 pups, 65 (64.4%) were sired by the breeding male of the group and 36 (35.6%) by other males. Neighbouring males sired altogether 28 pups (27.7%) (Fig. 2a). These males consisted of neighbouring breeding males (sired 21 pups or 20.8%), roamers (sired 6 pups or 5.9%) and one neighbouring philopatric male (sired 1 pup or 1.0%). Seven pups (6.9%) were sired by unknown males. Taking social group as the unit of analysis, significantly more extra-group young were sired by neighbouring males (3.0 ± 2.4 pups) than by unknown strange males (0.8 ± 1.4 pups; paired t_8 = 2.53, p = 0.035). Only one pup was sired (1.0%) by a natal philopatric male (Fig. 2a).

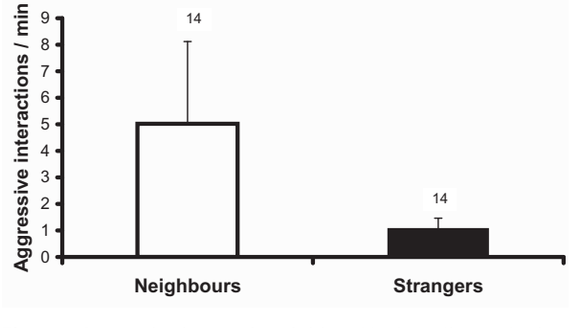

Figure 1 Aggressive interactions initiated by breeding males in neutral arena tests during the breeding season towards neighbouring breeding males or towards breeding males not neighbouring them ("strangers"). Mean, SE and sample sizes are shown. p = 0.001.

Comparison between young and old breeding females: indication for active female choice

In 6 of the 9 groups, some philopatric young females born at the start of the breeding season reproduced at the end of the breeding season. Altogether 14 of the 50 young females born between July and October 2005 bred, while 18 of the 20 old females born between September 2004 and June 2005 bred. Significantly more old than young females were reproductive (Fisher's Exact test, p < 0.0001). Young breeding females differed from the old breeding females in the pattern of extra-group paternities (Fig. 2b and 2c). The breeding males of the groups were the father of 79.6 ± 17.2% of the offspring of old females, but only of 12.5 ± 20.9% of the offspring of young females (paired t_5 = 6.624, p < 0.01; Fig 2b and 2c). Taking social group as the unit of analysis, significantly more extra-group young were sired by neighbouring males (1.8 ± 1.6 pups) than by unknown strange males (0.1 ± 0.3 pups) for old breeding females (paired t_8 = 3.162, p = 0.01), but not for young breeding females (1.2 ± 1.3 pups *versus* 0.8 ± 1.4 pups; paired t_8 = 1.51, p = 0.17).

Of the 14 young philopatric females that bred we could determine the father for 13 using the same microsatellites. For 12 of these females, the father was either still present as breeding male in their group (8 females), or their father was a neighbouring male that was still present (4 females). Only 2 of the 8 young females whose father was still the breeder of the group reproduced with him, while 18 of the 20 old breeding females reproduced with the breeding male of their group (Fisher's Exact Test, p < 0.002).

Discussion

In the present study we showed that breeding male striped mice were more aggressive during the breeding season than during the non-breeding season. Further, the neighbours of breeding males appear to pose a recognisable threat to the breeding male's confidence of paternity and direct fitness. This threat explains the occurrence of the nasty neighbour phenomenon in striped mice and the aggressive responses elicited when a breeding male encounters his neighbour.

Striped mice are territorial [20] and both breeders and philopatrics participate in territorial defence [22]. Here we showed that male breeders are more aggressive than male philopatrics that typically do not breed within their group (for a similar result in coyotes see [3]), though they might seek copulations with females from neighbouring groups. Additionally, breeding males were more aggressive towards strangers during the breeding season than during the non-breeding season. Together with a previous study showing that males are less territorial towards females than towards males [22], these results indicate

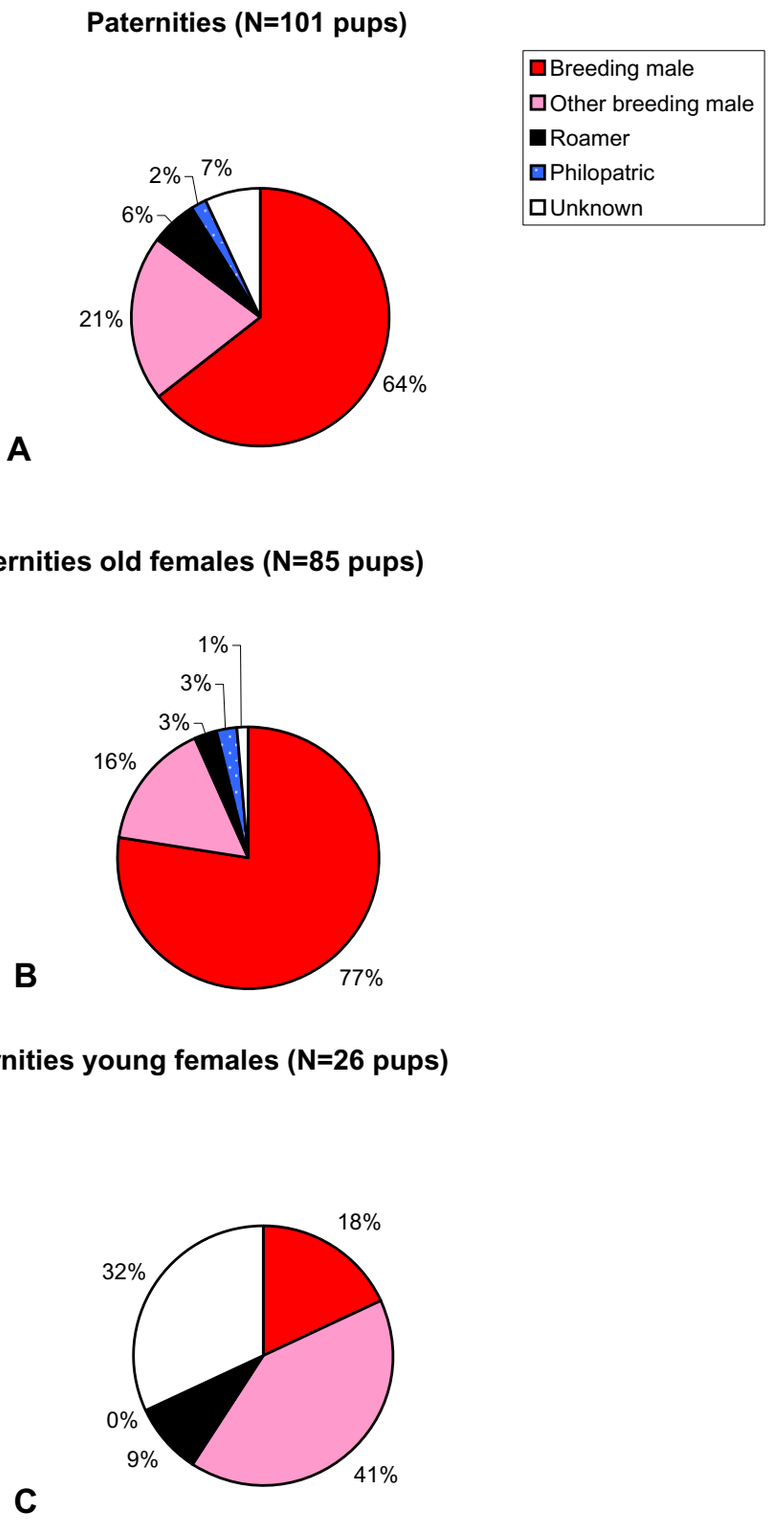

Figure 2 Paternity within groups by the breeding male of the group, neighbouring breeding males, roamers, philopatrics and unknown males, for A) entire groups, B) old breeding females, C) young philopatric females.

that male aggression occurs within the reproductive context. While year-round territoriality by all group members might act to protect resources such as food and shelter [22], the additional increase in aggression in male breeders is best explained as a mechanism to keep rival males from their territory and by this from their mating partners.

Male striped mice distinguish between males neighbouring them and strange males, indicating that they can differentiate between familiar and unfamiliar individuals or might even have the ability to recognize individuals (as described in other rodents: [31,32]. Our study provides growing evidence that the nasty neighbour phenomenon is more common than previously believed, as males were nearly five times more aggressive towards their neighbours than towards strangers. This indicates that male striped mice can alter their territorial behaviour which most likely depends on a pay-off asymmetry [33]: breeders have paternity to lose while philopatrics do not, and neighbours represent a stronger threat than strangers.

In territorial birds, neighbouring males often represent the greatest risk for loss of paternity to territory holders [34,35]. The same holds true for the striped mouse, where about 28% of pups are sired by neighbours. These were primarily neighbouring territorial males, but also solitary roaming males and to a much lesser extent philopatric males of neighbouring groups. On average, much more paternity was lost to territorial breeders than the other two categories of neighbouring males (Fig. 2). In another study we show that reproductive success of roamers is ten times smaller than that of territorial breeders, with philopatrics having even a hundred fold less reproductive success (Schradin and Lindholm, unpubl. data). In contrast, potential floating males that would be unfamiliar to striped mice played a minor role in extra-group paternity. In conclusion, neighbouring males and especially neighbouring territorial breeders induce high direct fitness costs for male breeders in the striped mouse, which use territorial aggression as a strategy to reduce these costs and defend paternity within their harems.

36% of litters had two or three fathers and the paternity share, an offspring-based measure of extra-group paternity independent of litter size [30] was 14%. High levels of multiple paternity are common in small mammals, both in polygynous [30] and socially monogamous species [36,37]. A paternity share of 14% is similar to the one observed in socially monogamous prairie voles (16%), but lower than the average of 21% reported for rodents and much lower than that of promiscuous rodent species (30-50%) [30]. Multiple mating by females is likely to represent active female choice [38] and can increase female fitness [39,40]. As females of one harem have synchronous oestrus but striped mice are solitary foragers [23], breeding males cannot continuously defend all their females, and the best strategy to defend paternity is to defend the territory and to keep neighbouring males away.

We found strong support for active female choice when comparing old breeding females born before the breeding season and their young adult philopatric daughters born at the start of the breeding season. Young females showed a much greater amount of extra-group paternity than old females (87% *versus* 20%). This was mainly due to paternity obtained by neighbouring males as well as unknown males. These data can best be explained by inbreeding avoidance. It is known from a captive study that female striped mice do not breed with the adult male with which they grew up, independent of whether this is their biological or foster father [41]. This indicates that familiarity is the mechanism by which female striped mice avoid inbreeding which is in accordance with our results: presence or absence of the biological father had no effect on young females' mate choice. Most young females chose a male outside of their natal group for mating, even if their biological father was a neighbouring male. The average tenure of breeding males of more than 2 months is long enough that inbreeding between them and their daughters could occur, as females can start breeding when 4-6 weeks old females [27]. These results can also explain why extra-group paternity was much greater than the paternity share in our study, even though it has been argued that both should measure the same [30]. The two measurements are only analogues when the primary male of the paternity share is also the social male of the group, but not when the primary male is from outside the group, as is the case for young breeding females. This might be important for many other cooperatively breeding species where subdominant females mate with males from outside the group, for example in meerkats (*Suricata suricatta*; [25]). In sum, active female choice might be a prerequisite for the high success rate of neighbouring males in obtaining extra-group paternity.

Breeding males might not be able to defend paternity of young breeding females which, in contrast to old breeding females, seem to seek copulations with other males, maybe to avoid inbreeding. In fact, it could increase the breeding male's fitness if his daughters breed with other males. However, the pattern that neighbours pose a greater risk than strangers was not created by the mate choice behaviour of young females. It is for the old females that the risk of losing paternity to neighbours versus strangers is the highest. Therefore we conclude that neighbours pose the highest risk for breeding males, and that strangers can have significant success with young females.

Conclusions

Müller & Manser [17] predicted that nasty neighbours are more common in social species that permanently stay

in large groups with intensive competition between neighbouring groups. In contrast to banded mongooses (*Mungos mungo*), which also show the nasty neighbour phenomenon and forage in groups [17], striped mice are solitary foragers [23]. Thus, striped mouse males would always only meet a single other male at one time [23] independent of whether this male would be a neighbour or a stranger. Thus, it is the threat of the single individual, not the entire neighbouring group to which male striped mice respond. The dear enemy phenomenon has been described as a kind of cooperation between non-kin [19], which requires that benefits must be greater than costs (b>c) to occur. Thus, a high risk of reduced fitness by neighbours will increase benefits of territoriality. We predict that with increasing rate of extra-pair fertilizations by neighbours, animal species will rather show the nasty neighbour instead of the dear enemy phenomenon, a hypothesis that could be tested in songbirds, where data on extra-pair fertilizations are available from many species (e.g. [42]).

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
C. Schradin designed the study, collected field samples and experiments and wrote the manuscript. C. Schneider collected data for the behavioural experiments. AKL did the genetic analyses and contributed to writing the manuscript. All authors have read and approved the final manuscript.

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References

1. Stamps J: **Territorial behavior: testing the assumptions.** *Adv Study Behav* 1994, **23:**173-232.
2. Davies NB, Houston AI: **Owners and satellites: the economics of territory defence in the pied wagtail, *Motacilla alba*.** *J Anim Ecol* 1981, **50:**157-180.
3. Gese EM: **Territorial defense by coyotes (*Canis latrans*) in Yellowstone National Park, Wyoming: who, how, where, and why.** *Can J Zool* 2001, **79:**980-987.
4. Taborsky M: **Broodcare helpers in the cichlid fish *Lamprologus brichardi:* their costs and benefits.** *Anim Behav* 1984, **32:**1236-1252.
5. Ewald PW, Orians GH: **Effects of resource depression on use of inexpensive and escalated aggressive behavior: experimental tests using anna hummingbirds.** *Behav Ecol Sociobiol* 1983, **12:**95-101.
6. Cant MA, Otali E, Mwangyhya F: **Fighting and mating between groups in a cooperatively breeding mammal, the banded mongoose.** *Ethology* 2002, **108:**541-555.
7. Schradin C, Lamprecht J: **Female-biased immigration and male peace keeping in groups of the shell-dwelling cichlid fish *Neolamprologus multifasciatus*.** *Behav Ecol Sociobiol* 2000, **48:**236-242.
8. Putland DA, Goldizen AW: **Territorial behaviour in the Tasmanian native hen: group and individual performance.** *Anim Behav* 1998, **56:**1455-1463.
9. Lazaro-Perea C: **Intergroup interactions in wild common marmosets, *Calithrix jacchus*: territorial defence and asessment of neighbours.** *Anim Behav* 2001, **62:**11-21.
10. Baker AJ, Dietz JM: **Immigration in wild groups of golden lion tamarins *(Leontopithecus rosalia).*** *Am J Primatol* 1996, **38:**47-56.
11. Anzenberger G: **How stranger encounters of common marmosets (*Callithrix jacchus*) are influenced by family members: the quality of behaviour.** *Folia Primatol* 1985, **45:**204-224.
12. Gray SJ, Jensen SP, Hurst JL: **Effects of resource distribution on activity and territory defence in house mice, *Mus domesticus*.** *Anim Behav* 2002, **63:**531-539.
13. Palanza P, Re L, Mainardi D, Brain PF, Parmigiani S: **Male and female competitive strategies of wild house mice pairs (*Mus musculus domesticus*) confronted with intruders of different sex and age in artificial territories.** *Behaviour* 1996, **133:**863-882.
14. Trivers RL: **Parental investment and sexual selection.** In *Sexual Selection and the Descent of Man* Edited by: Campbell B. Chicago: Aldine; 1972:136-179.
15. Temeles EJ: **The role of neighbours in territorial systems: when are they 'dear enemies'?** *Anim Behav* 1994, **47:**339-350.
16. Whiting MJ: **When to be neighbourly: differential agonistic responses in the lizard *Platysaurus broadleyi*.** *Behav Ecol Sociobiol* 1999, **46:**210-214.
17. Müller CA, Manser MB: **Nasty neighbours rather than dear enemies in a social carnivore.** *Proc R Soc B* 2007, **274:**959-965.
18. Brunton DH, Evans B, Cope T, Ji W: **A test of the dear enemy hypothesis in female New Zealand bellbirds (*Anthornis melanura*): female neighbors as threats.** *Behav Ecol* 2008, **19:**791-798.
19. Olendorf R, Getty T, Scribner K, Robinson SK: **Male red-winged blackbirds distrust unreliable and sexually attractive neighbours.** *Proc R Soc Lond B* 2004, **271:**1033-1038.
20. Schradin C, Pillay N: **The striped mouse (*Rhabdomys pumilio*) from the succulent karoo of South Africa: A territorial group living solitary forager with communal breeding and helpers at the nest.** *J Comp Psychol* 2004, **118:**37-47.
21. Schradin C, Scantlebury M, Pillay N, König B: **Testosterone levels in dominant sociable males are lower than in solitary roamers: Physiological differences between three male reproductive tactics in a sociably flexible mammal.** *Am Nat* 2009, **173:**376-388.
22. Schradin C: **Territorial defense in a group living solitary forager: who, where, against whom?** *Behav Ecol Sociobiol* 2004, **55:**439-446.
23. Schradin C: **Whole day follows of the striped mouse.** *J Ethol* 2006, **24:**37-43.
24. Schradin C, Pillay N: **Demography of the striped mouse (*Rhabdomys pumilio*) in the succulent karoo.** *Mammal Biol* 2005, **70:**84-92.
25. Young AJ, Spong G, Clutton-Brock T: **Subordinate male meerkats prospect for extra-group paternity: alternative reproductive tactics in a cooperative mammal.** *Proc R Soc Lond B* 2007, **274:**1603-1609.
26. Double MC, Cockburn A: **Subordinate superb fairy-wrens (Malurus cyaneus) parasitize the reproductive success of attractive dominant males.** *Proc Roy Soc Lond B* 2003, **270:**379-384.
27. Schradin C, Pillay N: **Intraspecific variation in the spatial and social organization of the African striped mouse.** *J Mammal* 2005, **86:**99-107.
28. Perrin MR, Ercoli C, Dempster ER: **The role of agonistic behaviour in the population of two syntopic African grassland rodents, the striped mouse *Rhabdomys pumilio* (Sparrman 1784) and the multimammate mouse *Mastomys natalensis* (A. Smith 1834) (Mammalia Rodentia).** *Trop Zool* 2001, **14:**7-29.

29. Teschke M, Mukabayire O, Wiehe T, Tautz D: **Identification of selective sweeps in closely related populations of the house mouse based on microsatellite scans.** *Genetics* 2008, **180:**1537-1545.
30. Eccard JA, Wolf JBW: **Effects of brood size on multiple-paternity rates: a case for [']paternity share' as an offspring-based estimate.** *Anim Behav* 2009, **78:**563-571.
31. Hurst JL, Payne CE, Nevison CM, Marie AD, Humphries RE, Robertson DHL, Cavaggioni A, Beynon RJ: **Individual recognition in mice mediated by major urinary proteins.** *Nature* 2001, **414:**631-633.
32. Gheusi G, Goodall G, Dantzer R: **Individually distinctive odours represent individual conspecifics in rats.** *Anim Behav* 1997, **53:**935-944.
33. Maynard Smith J, Parker GA: **The logic of asymmetric contests.** *Anim Behav* 1976, **24:**159-175.
34. Gibbs HL, Weatherhead PJ, Boag PT, White BN, Tabak LM, Hoysak DJ: **Realized reproductive success of polygynous red-winged blackbirds revelaed by DNA markers.** *Science* 1990, **250:**1394-1397.
35. Richardson DS, Jury FL, Blaakmeer K, Komdeur J, Burke T: **Parentage assignment and extra-group paternity in a cooperative breeder: the Seychelles warbler (*Acrocephalus sechellensis*).** *Molec Ecol* 2001, **10:**2263-2273.
36. Ophir AG, Phelps SM, Sorin AB, Wolff JO: **Social but not genetic monogamy is associated with greater breeding success in prairie voles.** *Anim Behav* 2008, **75:**1143-1154.
37. Solomon NG, Keane B, Knoch LR, Hogan PJ: **Multiple paternity in socially monogamous prairie voles (*Microtus ochrogaster*).** *Can J Zool* 2004, **82:**1667-1671.
38. Klemme I, Eccard JA, Ylönen H: **Do female bank voles (*Clethrionomys glareolus*) mate multiply to improve in previous mates?** *Behav Ecol Sociobiol* 2006, **60:**415-421.
39. Keil A, Sachser N: **Reproductive benefits from female promiscuous mating in a small mammal.** *Ethology* 1998, **104:**897-903.
40. Klemme I, Ylönen H, Eccard JA: **Long-term fitness benefits of polyandry in a small mammal, the bank vole Clethrionomys glareolus.** *Proc Roy Soc Lond B* 2008, **275:**1095-1100.
41. Pillay N: **Father-daughter recognition and inbreeding avoidance in the striped mouse, *Rhabdomys pumilio*.** *Mamm Biol* 2002, **67:**212-218.
42. Griffith SC, Owens IPF, Thuman KA: **Extra pair paternity in birds: a review of interspecific variation and adaptive function.** *Molec Ecol* 2002, **11:**2195-2212.

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Chapter 14

Development of polymorphic microsatellite markers for the livebearing fish *Poecilia parae*

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PERMANENT GENETIC RESOURCES

Development of polymorphic microsatellite markers for the livebearing fish *Poecilia parae*

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Abstract

We developed 16 novel polymorphic tetranucleotide microsatellite markers for *Poecilia parae*, a livebearing fish used in evolutionary studies because of its Y-linked colour and size polymorphism. A set of 199 clones was sequenced out of an enriched genomic library, and we achieved an enrichment efficiency of nearly 80%. Primers were designed for 16 pure repeats, and 59 *P. parae* were screened for polymorphism. Cross-amplification was tested on *Poecilia picta* and *Poecilia reticulata*, the guppy. The new microsatellite markers showed an exceptionally high allelic diversity and low stutter formation, proving their suitability for a broad range of applications in these species.

Keywords: genetic marker, microsatellites, paternity testing, *Poecilia parae*, sexual selection

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Colour polymorphisms have long been used as convenient genetic markers in studies of natural and sexual selection (e.g. Cain & Sheppard 1950). Species in which colour polymorphisms are sex-limited, such as in several poeciliid fishes (Lindholm & Breden 2002), can be particularly useful for studies of selection within that sex. Polymorphisms in males of the livebearing poeciliid fish, *Poecilia parae*, are ideal for such studies, as males occur in up to five discrete colour morphs within the same population, with an associated size polymorphism (Lindholm *et al*. 2004). Colour morphs and adult body size in males are paternally inherited, consistent with linkage to the Y-chromosome (Lindholm *et al*. 2004; Lindholm *et al*. 2006). The frequency of each colour morph before and after a selection episode can be easily scored and used for estimates of changes in gene frequency. Colour morphs can be used as markers of paternity for one sex, as sons always develop the colour morph of their sires upon maturity. However, the use of adult male colour patterns as genetic markers for paternity is limited unless only one male of a given morph is a potential sire, and one only wishes to score paternity of sons that survive until maturity and not daughters or juveniles. To circumvent this limitation, we developed microsatellite markers for *P. parae* and tested our markers for use with the closely related *Poecilia picta* and the well-studied guppy, *Poecilia reticulata*.

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Poecilia parae were sampled in 2002 in Guyana from Patentia (north side of Demerera River; population 1), Georgetown (south side of Demerara River; population 2), and Rosignol (west side of Berbice River; population 3). *P. picta* were sampled in Georgetown in 2000, while *P. reticulata* were sampled in 2002 from Georgetown. Total genomic DNA was isolated by salt extraction from muscle near the base of the tail and quantified with an ND-1000 spectrophotometer (NanoDrop). Ten micrograms of genomic DNA from a male *P. parae* individual was digested with *Nhe*I and *Alu*I (both New England Biolabs) and size-selected for fragment sizes between 400 and 1200 base pairs. The DNA fragments were processed with mung bean nuclease (New England Biolabs) and CIP (New England Biolabs), followed by ligation of an SNX linker to both ends (Hamilton *et al*. 1999). Enrichments were performed by hybridization with biotinylated oligonucleotide probes [$(GACA)_7$ $(GATA)_7$ $(GATC)_7$ and $(CA)_{24}$] containing a 3′-dideoxy nucleotide to prevent probes from acting as primers in the subsequent polymerase chain reaction (PCR) (Koblizkova *et al*. 1998). M-280 streptavidin-coated Dynabeads (Dynal) were used to capture the biotinylated oligos. The enriched fragments were digested with *Nhe*I (New England Biolabs) and ligated into an *Xba*I-cut pGEM-3Z vector (Promega). The ligation was performed with T4 DNA Ligase (New

England Biolabs) together with *Nhe*I (New England Biolabs) to prevent dimerization of fragments. A competent *Escherichia coli* strain JM109 (> 10^8 cfu/μg, Promega) was transformed with 30 ng of vector-ligated DNA fragments according to the manufacturer's instructions, and plasmids were isolated with a Perfectprep Plasmid Mini Kit (Eppendorf). Cycle sequencing was performed with 300–500 ng of plasmid DNA on a 3730 DNA sequencer (Applied Biosystems) using BigDye Terminator version 3.1 sequencing chemistry (Applied Biosystems) and the following sequencing primers: forward: TTGTAAAACGACGGCCAGTGAAT; reverse: TTTATGCTTCCGGCTCGTATGTTG. Clones were sequenced in both directions whenever it was necessary and manually screened for any repeats. The SEQMAN program of the LASERGENE 6 software package (DNASTAR) was used to align forward and reverse sequences and to remove redundant vector and linker sequences. The PRIMERSELECT program of the LASERGENE package was used to design PCR primers for sequences containing pure repeats. For large-scale screening, forward primers for 16 loci were labelled with fluorescent dyes (Applied Biosystems, Table 1), and 59 *P. parae* (35 males, 24 females) from three different populations were tested. Cross-amplifications were performed with 19 *P. picta* (10 males, nine females) and 20 *P. reticulata* (16 males, four females). PCRs contained 20 ng of template DNA, 5 μL of 2× Multiplex PCR Master Mix (QIAGEN), 1 μL 10× primer mix (between 0.05 μM and 0.8 μM final concentration per primer) and double-distilled water to 10 μL volume. The PCR amplifications were performed in a PTC-220 thermocycler (MJ Research) with the following profile: initial activation at 95 °C for 15 min, 30 cycles of 30 s at 94 °C, 90 s at 64 °C (multiplex reaction 1; Table 1) or 62 °C (multiplex reaction 2) and 60 s at 72 °C, followed by a final extension step of 30 min at 60 °C to promote complete adenylation of the PCR products. The PCR products were diluted 20–40 times with double-distilled water. One microlitre of the diluted PCR product was added to 10 μl of Hi-Di formamide (Applied Biosystems) containing 0.07 μL of GS-500LIZ size standard (Applied Biosystems) and denatured for 3 min at 95 °C. The samples were run on a 3730 DNA sequencer and analysed with the GENEMAPPER 4.0 software (Applied Biosystems). The statistical analyses were performed with MStools version 3.1 add-in to Microsoft Excel (Park 2001) and GENEPOP version 4.0 software (Rousset 2008).

A total of 48 and 199 positive clones that were expected to contain di- and tetranucleotide repeats, respectively, were sequenced. Enrichment efficiency for both di- and tetranucleotide motifs proved to be very high, with about 80% of all clone sequences showing a repeat-like structure. Out of the available repeats, only 16 of the longest and purest tetranucleotide repeats were tested for polymorphism. Allelic diversity was generally very high, with some markers showing as many as 22 alleles in a single

Table 1 Characterization of the isolated *Poecilia parae* microsatellite markers in three different populations [Repeat, longest uninterrupted repeat stretch in the cloned sequence; ASR, allele size range; Pop., population; N, number of individuals; N_A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; P, P value of probability test for deviation from Hardy–Weinberg equilibrium (HWE)]

Locus name GenBank no.	Primer sequences (5′–3′)	Label	Multiplex*	Repeat	ASR (bp)	Pop.	N	N_A	H_O	H_E	P	Comment
PP_GATA_5	F: TGAGGTGTGGGAGACTGAACTAC	VIC	1	$(GATA)_{18}$	130–216	1	19	9	0.684	0.805	0.2097	
EU293044	R: GTGTCCAGGCATGTCAGATAAAT	—				2	20	10	0.900	0.869	0.3612	
						3	16	13	0.625	0.911	0.0026†	
PP_GATA_A3	F: TAAACCGGGTCATCACAAACAAGAG	6-FAM	1	$(GATA)_{26}$	291–446	1	19	22	0.895	0.963	0.2917	
EU293045	R: TGCCAAAGACAAAAGAGACAACTGC	—				2	19	22	0.947	0.954	0.4769	
						3	14	15	0.929	0.934	0.4867	
PP_GATG_B4	F: CACCGTTGTAATGTTAATGTGACAC	PET	1	$(GATG)_8$	223–271	1	19	11	0.789	0.855	0.8974	
EU293046	R: ACTCTGACGGGCAGGAAGTC	—				2	21	10	0.762	0.844	0.0491	
						3	14	9	0.929	0.870	0.8771	
PP_GATG_C4	F: TTCCTCCCACAGTCCAAAAACAT	PET	2	$(GATG)_{44}$	266–420	1	19	13	0.789	0.905	0.0535	
EU293047	R: AAGACAAATGCGGCACAAGAGTT	—				2	20	13	0.850	0.882	0.8153	
						3	13	9	0.846	0.877	0.4449	
PP_GACA_C7	F: ATTCCCTTATGTTAATCTTTCAGTC	VIC	2	$(GACA)_{10}$	112–132	1	20	3	0.400	0.417	0.5228	
EU293048	R: ACACCTCTGTGCCATCTCAC	—				2	20	3	0.200	0.188	1.0000	
						3	14	2	0.214	0.198	1.0000	

Table 1 *Continued*

Locus name GenBank no.	Primer sequences (5′–3′)	Label	Multiplex*	Repeat	ASR (bp)	Pop.	*N*	N_A	H_O	H_E	*P*	Comment
PP_GATA_D9	F: GGAGAACATCCCGACAATGCTGTA	PET	1	$(GACA)_9$	361–580	1	18	13	0.167	0.910	0.0000†	Possibly
EU293051	R: CCTGCGTGGAAGACTGGTGACTTA	—		$(GATA)_{18}$		2	21	16	0.476	0.937	0.0000†	X-linked
						3	14	13	0.286	0.931	0.0000†	
PP_GATG_D10	F: CGCCTGGAACGTTAGCTGTAGATA	PET	1	$(GATG)_{11}$	128–172	1	19	7	0.947	0.842	0.6247	
EU293049	R: CCTTTCAAAAATTTCATTTGGACTT	—				2	20	7	0.800	0.799	0.7228	
						3	17	5	0.824	0.811	0.3187	
PP_GACA_D11	F: TGAAATCAAGGGGGAAAATCTG	NED	2	$(GACA)_{26}$	190–356	1	19	17	0.895	0.927	0.2646	
EU293050	R: ACCAGCCAACCAGGAACACATA	—		$(GATA)_{17}$		2	21	22	0.952	0.962	0.7839	
						3	14	18	0.929	0.963	0.6144	
PP_GATG_E5	F: ATGGCCGAGTCCAAGACCTGAG	PET	2	$(GATG)_{20}$	163–243	1	19	12	0.842	0.892	0.3150	
EU293052	R: CCGACCCGCTTTTAATGTTGAA	—				2	20	13	0.950	0.924	0.7064	
						3	13	9	0.846	0.895	0.5836	
PP_GATG_F4	F: GCTGCCGTTTCATTATTTGCTTCA	6-FAM	2	$(GATG)_{14}$	230–364	1	19	10	0.842	0.855	0.5890	
EU293053	R: ACAGTCTGGAACGGAAACTTCTGGA	—				2	20	12	0.850	0.849	0.7829	
						3	13	13	0.923	0.945	0.3199	
PP_GATA_G7	F: GCAGGCTGCACAGTGTTCG	VIC	2	$(GATA)_7$	182–250	1	20	7	0.800	0.796	0.7894	
EU293054	R: CAGCCGTTTCCAATTAATGAGTCA	—				2	21	10	0.810	0.843	0.3112	
						3	13	9	0.692	0.858	0.1363	
PP_GATA_H2	F: TGAATACCTCAAAAATCATGTCTGTTT	VIC	2	$(GATA)_{25}$	321–399	1	19	11	0.789	0.865	0.6289	
EU293055	R: TGAATAGGTGCTGTCATTTTCTTTTC	—				2	19	11	1.000	0.908	0.8920	
						3	13	7	0.615	0.837	0.0755	
PP_GATG_H3	F: ACTATATCTGCCGGTTTGTTTACG	NED	1	$(GATG)_{28}$	182–266	1	15	5	0.267	0.733	0.0000†	
EU293056	R: GAGGCCCTGGGTTACTGTCA	—				2	15	8	0.467	0.823	0.0002†	
						3	13	8	0.308	0.815	0.0005†	
PP_GATG_H4	F: TGGCGACCTATCCAGGGTGAC	VIC	1	$(GATG)_{19}$	226–394	1	19	11	0.737	0.886	0.1097	
EU293057	R: AGTTGGCTGACAGGGATTTGAATG	—				2	21	11	0.714	0.812	0.3405	
						3	14	10	0.786	0.897	0.1343	
PP_GATA_T1	F: GCCCAACGCCATTTATTACA	6-FAM	1	$(GATA)_{28}$	152–238	1	20	13	0.850	0.901	0.1107	
EU293058	R: GAGGTGGGCATTTATATCATTTATG	—				2	21	17	0.857	0.941	0.2492	
						3	15	12	0.800	0.897	0.1727	
PP_GACA_W1	F: TTATTATTGTTGCAAACCCAAAAAT	6-FAM	2	$(GACA)_5$	109–129	1	19	4	0.263	0.644	0.0001†	Possibly
EU293059	R: TACGACGATTGTCACTGACTGTAAG	—				2	20	4	0.200	0.406	0.0122	X-linked
						3	14	6	0.571	0.778	0.1266	

*Annealing temperature for primer pairs was 64 °C for multiplex 1 °C and 62 °C for multiplex 2.
†Significant deviations from HWE after sequential Bonferroni correction (Rice 1989).

Table 2 Results of cross-amplification of the *Poecilia parae* markers in *Poecilia picta* and *Poecilia reticulata* (ASR, allele size range; *N*, number of individuals; N_A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity)

	P. picta					*P. reticulata*				
Locus name	*N*	ASR (bp)	N_A	H_O	H_E	*N*	ASR (bp)	N_A	H_O	H_E
PP_GATA_5	19	224–316	17	0.947	0.943	20	176–332	19	0.850	0.935
PP_GATA_A3	—	—	—	—	—	19	279–287	2	0.158	0.149
PP_GACA_C7	18	108–108	1	0.000	0.000	9	108–136	5	1.000	0.810
PP_GATA_D9	—	—	—	—	—	10	316–324	3	0.100*	0.700
PP_GATG_D10	19	120–120	1	0.000	0.000	10	128–136	3	0.300	0.563
PP_GATG_E5	16	175–179	2	0.188	0.417	—	—	—	—	—
PP_GATG_F4	9	433–581	10	0.889	0.922	7	407–591	12	1.000	0.978
PP_GATA_G7	18	182–210	8	0.722	0.778	—	—	—	—	—
PP_GATA_H2	13	321–369	9	0.769	0.886	7	285–389	11	1.000	0.956
PP_GATG_H3	19	194–234	8	0.842	0.852	—	—	—	—	—
PP_GATG_H4	19	352–388	9	0.737	0.832	19	447–529	24	0.737*	0.970

—no PCR product detectable under standard PCR conditions.
*significant deviations from HWE after sequential Bonferroni correction (Rice 1989).

population (Table 1). Expected heterozygosity over all populations and loci was calculated as 0.850 (± 0.043 SD) and mean number of alleles as 17.94 (± 9.41 SD). The loci PP_GATA_5, PP_GATA_D9, PP_GATG_H3 and PP_GACA_W1 deviated significantly from Hardy–Weinberg equilibrium (HWE) for some or all populations, and all showed a deficiency in heterozygous genotypes. Locus PP_GATA_5 showed a deficiency of heterozygous genotypes only for population 3. PP_GACA_W1 and PP_GATA_D9 are most likely located on the X chromosome, since no male heterozygous genotypes could be observed. Both loci are in HWE if only the female samples are used in the analysis (data not shown). For the locus PP_GATG_H3, a high frequency of null alleles could be an explanation for the deviations from HWE. The same marker was in HWE when cross-amplified in the *P. picta* samples. No significant linkage disequilibrium could be observed with any pair of loci in the tested *P. parae* samples.

Cross-amplification of the newly isolated microsatellite markers in *P. picta* and *P. reticulata* was performed with no further optimization with a success rate of 71% and 53%, respectively, reflecting the greater phylogenetic distance of *P. parae* to *P. reticulata* compared to *P. picta* (Breden *et al*. 1999). Some of the markers did not show polymorphism in the other species (Table 2).

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References

Breden F, Ptacek M, Rashed M, Taphorn D, de Figueiredo CA (1999) Molecular phylogeny of the live-bearing fish genus *Poecilia* (Cyprinodontiformes: Poeciliidae). *Molecular Phylogenetics and Evolution*, **12**, 95–104.

Cain AJ, Sheppard PM (1950) Selection in the polymorphic land snail *Cepaea nemoralis*. *Heredity*, **4** (3), 275–294.

Hamilton MB, Pincus EL, Di Fiore A, Fleischer RC (1999) Universal linker and ligation procedures for construction of genomic DNA libraries enriched for microsatellites. *BioTechniques*, **27** (3), 500–507.

Koblizkova A, Dolezel J, Macas J (1998) Subtraction with 3′ modified oligonucleotides eliminates amplification artifacts in DNA libraries enriched for microsatellites. *BioTechniques*, **25** (1), 32–38.

Lindholm A, Breden F (2002) Sex chromosomes and sexual selection in poeciliid fishes. *American Naturalist*, **160**, S143–S224.

Lindholm AK, Brooks R, Breden F (2004) Extreme polymorphism in a Y-linked sexually selected trait. *Heredity*, **92** (3), 156–162.

Lindholm AK, Hunt J, Brooks R (2006) Where do all the maternal effects go? Variation in offspring body size throughout ontogeny in the live-bearing fish *Poecilia parae*. *Biology Letters*, **2**, 586–589.

Park SDE (2001) *Trypanotolerance in West African cattle and the population genetic effects of selection*. PhD Thesis, University of Dublin.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43** (1), 223–225.

Rousset F (2008) GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources*, **8** (1), 103–106.

Chapter 15

Direct selection on male attractiveness and female preference fails to produce a response

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Direct selection on male attractiveness and female preference fails to produce a response

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Abstract

Background: Theoretical studies suggest that direct and indirect selection have the potential to cause substantial evolutionary change in female mate choice. Similarly, sexual selection is considered a strong force in the evolution of male attractiveness and the exaggeration of secondary sexual traits. Few studies have, however, directly tested how female mate choice and male attractiveness respond to selection. Here we report the results of a selection experiment in which we selected directly on female mating preference for attractive males and, independently, on male attractiveness in the guppy, *Poecilia reticulata*. We measured the direct and correlated responses of female mate choice and male attractiveness to selection and the correlated responses of male ornamental traits, female fecundity and adult male and female survival.

Results: Surprisingly, neither female mate choice nor male attractiveness responded significantly to direct or to indirect selection. Fecundity did differ significantly among lines in a way that suggests a possible sexually-antagonistic cost to male attractiveness.

Conclusions: The opportunity for evolutionary change in female mate choice and male attractiveness may be much smaller than predicted by current theory, and may thus have important consequences for how we understand the evolution of female mate choice and male attractiveness. We discuss a number of factors that may have constrained the response of female choice and male attractiveness to selection, including low heritabilities, low levels of genetic (co)variation in the multivariate direction of selection, sexually-antagonistic constraint on sexual selection and the "environmental covariance hypothesis".

Background

Female mate choice imposes sexual selection on males and is responsible for the evolution of elaborate male ornamentation. Choosy females may benefit adaptively from their choice, both directly (males provide benefits that increase female fecundity) and indirectly (offspring inherit genes that confer superior fitness from the chosen male) [1]. Thus female mate choice and male attractiveness are traits that are causally linked and that mutually influence one another's evolutionary fates. Despite a sophisticated body of theory and substantial empirical research on the mechanics of choice and the benefits that

Table 1: The number of males (M) or females (F) measured (in parentheses) and selected in each generation of selection. The selection treatments include up attractiveness (AT), down attractiveness (UN), up preference for attractive males (PR) and control (CO).

Generation		Block 1 selected (measured)					Block 2 selected (measured)				
		AT	UN	PR	CO	Date	AT	UN	PR	CO	Date
Parental	M	50 (100)	50 (100)	50 (50)	50 (50)	Feb 2000	50 (100)	50 (100)	50 (50)	50 (50)	May 2000
	F	50 (50)	50 (50)	50 (100)	50 (50)		50 (50)	50 (50)	50 (100)	50 (50)	
F1	M	38 (57)	38 (57)	38 (38)	38 (38)	Mar 2001	35 (57)	35 (57)	35 (35)	35 (35)	May 2001
	F	40 (40)	40 (40)	40 (76)	40 (40)		40 (40)	40 (40)	40 (76)	40 (40)	
F2	M	50 (84)	50 (90)	50 (50)	50 (50)	Oct 2001	50 (77)	50 (90)	50 (50)	50 (50)	Jan 2002
	F	50 (50)	50 (50)	50 (95)	50 (50)		50 (50)	50 (50)	50 (114)	50 (50)	

males may provide to females [2-4], there are few empirical measures of how mate choice and attractiveness respond to selection. Here we describe an experiment in which we selected directly on female mate choice and on male attractiveness in guppies (*Poecilia reticulata*), and measured the change in these traits. We also measured the indirect responses to selection of the male ornaments on which females are thought to base their choice, and of life-history traits that may be genetically correlated with attractiveness and thus provide indirect benefits.

Selection acting on female mate choice

Natural selection acts directly on female mate choice due to the direct benefits and costs associated with choosing a mate [2,3]. Examples of direct benefits include increased fertility, fecundity, resource provision, parental provision, breeding territories or a reduction in predation and harassment risks [3]. Natural selection may oppose the evolution of female mate choice if, for example, searching for and evaluating mates results in increased energy expenditure or predation risks [3,5].

Female mate choice may also be subject to indirect selection when there are genetic correlations between mate choice and other fitness components exposed to selection [1,6,7]. Indirect selection requires that there is additive genetic variation underlying female mate choice and overall fitness, and that the male displays and ornaments indicate this variation in fitness [7,8]. Thus, females benefit indirectly from being choosy because of the superior genes that chosen males pass on to the mutual offspring. Such a process favours the spread of "choosiness" genes leading to the evolution of an adaptive preference for male traits that indicate increased genetic quality (i.e. breeding value for total fitness) [7,8].

Despite the many studies on the direct and indirect benefits of female mate choice, the potential of the two processes to effect the evolution of mate choice has yet to be directly demonstrated [4]. It is particularly important that empiricists attempt such tests – logistically demanding as they may be – because recent theoretical treatments have differed as to the likely strength and relative importance of direct and indirect selection on mate choice [9-11].

Selection acting on male attractiveness

In many species, females can only differentiate between potential mates based on the secondary sexual traits of the individual males. If phenotypic variation in male displays or ornaments exists, then any female biases toward mating with males of certain phenotypes will lead to differential male mating success [3,4]. If any of this phenotypic variation is heritable, differential mating success will, in turn, lead to the exaggeration of the ornament by sexual selection [1,3,12]. Several studies have demonstrated sexual selection operating on male ornamental and display traits (see [3] for an extensive review), the presence of substantial additive genetic variation in these traits [13,14] and in male attractiveness itself [15].

Artificial selection experiments are useful for understanding how traits respond directly to selection and indirectly to selection on correlated traits. There have been several recent demonstrations that attractive male display traits respond, in relatively few generations, to direct artificial selection [16-19]. Despite this wealth of evidence, there are no direct demonstrations that sexual selection on male attractiveness causes measurable evolutionary change in attractiveness and the ornamental and display traits that underpin it.

Here we present the results of an experiment in which we selected directly on male attractiveness and on female preference for attractive males in the guppy, *Poecilia reticulata*. Female guppies actively choose males based on highly distinctive and polymorphic ornamentation patterns, and both males and females mate multiply [20]. Accordingly, there is the strong potential for mate choice evolution and sexual selection. Importantly, the guppy has already proved to be a highly suitable organism for understanding the evolution of mate choice by indirect selection due to its non-resource based mating system

Table 2: Realised heritabilities of directly selected traits. Selection intensity is the cumulative intensity over the three generations, in units of phenotypic standard deviation. The response to selection is the divergence in the selection trait from the control line means, in control phenotypic standard deviations. Means and standard errors (SE) are included for the responses to selection and realized heritabilities. Selection intensities and responses in the up direction will have a positive sign, in the down direction a negative sign.

Selection treatment	Selection intensities		Selection response				Realized heritabilities			
	Block 1	Block 2	Block 1	Block 2	Mean	SE	Block 1	Block 2	Mean	SE
Up attractiveness (AT)	1.000	0.904	0.009	-0.044	-0.017	0.019	0.009	-0.048	-0.020	0.029
Down attractiveness (UN)	-1.030	-0.927	0.003	0.055	0.0288	0.018	-0.003	-0.059	-0.031	0.028
Up preference for attractive males (PR)	1.178	0.994	0.072	-0.191	-0.060	0.093	0.061	-0.192	-0.066	0.127

[15,16,19,21-23]. There is also substantial demonstrated additive genetic variation in male attractiveness and mating success [15,24] and in the ornamental traits on which attractiveness is based [15,25].

We directly selected the most attractive males (up attractiveness treatment), the least attractive males (down attractiveness treatment) and the females showing the strongest preference for attractive males (up preference treatment) and compared changes in these lines with control lines that experienced no selection. Instead of selecting on specific male traits and female preference functions, we selected individuals based on the results of behavioural mate choice trials. We thus imitated the actual processes involved in sexual selection and preference evolution in natural populations. We attempt to answer three important and related questions. First, how does female mate choice evolve under direct selection and as a correlated response to selection on male attractiveness? Second, how does male attractiveness evolve under direct selection and a correlated response to selection on female preferences? Third, how do other traits, especially those thought to determine attractiveness and those associated with other fitness components, evolve as a correlated response?

Results

Neither male attractiveness nor female preference for attractive males responded significantly to direct selection (Table 2). The realized heritabilities in both attractiveness and preference are inconsistent in magnitude and direction among lines such that the mean heritability estimates are small, and not significantly different from zero (Table 2).

More generally, there were no significant differences among treatments in any of the male attractiveness, female choosiness or female preference function measures (Table 3). Two of the three male ornaments most strongly associated with male attractiveness in the Alligator creek guppy population (tail area and the area of orange colouration [15]) also did not differ significantly between selection treatments, but the third (area of iridescence) did (Table 3). Furthermore, the total number of spots differed significantly among treatments (Table 3). Post-hoc comparisons revealed that iridescence was significantly lower in the down attractiveness treatment (compared with all other treatments) and that spot numbers were significantly lower in the down attractiveness line than in the up preference line. Taken together these results indicate that although preference and attractiveness did not respond to direct or indirect selection, some ornamental traits did. Moreover, it seems that these changes were largely due to a decrease in attractive ornaments (iridescence and spot number – an index of overall pattern complexity) in the down attractiveness line.

Survival did not differ significantly between the selection treatments (one-way ANOVA $F_{3,4} = 0.98$, $P = 0.492$), and does not appear to have responded indirectly to selection on either male attractiveness or female preference for attractive males. Selection did, however, result in correlated changes in at least one fitness component: female fecundity in the down attractiveness treatment was significantly higher than all other treatments, and in the up preference line it was significantly lower than all other treatments ($F_{3,4}= 69.00$, $P = 0.000$, and Tukey's post-hoc comparisons).

Discussion

Neither female mate choice nor male attractiveness responded significantly to the selection that we imposed, in contrast to the predictions of theoretical models of sexual selection and mate choice evolution. Direct and indirect selection on mate choice are expected to cause substantial evolutionary change [4,9-11,26]. Similarly, sexual selection resulting from female choice of attractive males is widely understood to cause the evolution of male attractiveness and the exaggeration of secondary sexual traits [3,12]. We spend the remainder of this paper considering why we failed to detect significant responses to three generations of selection on male attractiveness and female

Table 3: Nested analysis of variance comparing traits between selection treatments and between blocks nested within treatment. The means used in the analysis have been standardised for the effect of block.

Measured character	Selection treatment			Block within treatment		
	df	F	P	Df	F	P
PARTITIONED-AQUARIA BEHAVIOUR TRIALS						
Male attractiveness	3, 4	0.31	0.820	4, 444	0.21	0.935
Female preference for attractive males	3, 4	0.93	0.505	4, 444	0.31	0.869
Female responsiveness	3, 4	2.32	0.217	4, 444	1.30	0.268
Female discrimination	3, 4	0.22	0.881	4, 444	1.11	0.351
OPEN-AQUARIA BEHAVIOUR TRIALS						
Male attractiveness	3, 4	2.85	0.169	4, 376	0.47	0.760
Female responsiveness	3, 4	0.51	0.694	4, 184	0.91	0.459
MALE ORNAMENTATION						
Body size	3, 4	1.09	0.451	4, 444	6.49	0.000
Tail size	3, 4	1.67	0.309	4, 444	2.29	0.059
Black	3, 4	0.70	0.598	4, 444	1.52	0.194
Fuzzy black	3, 4	1.55	0.332	4, 444	0.82	0.513
Orange	3, 4	1.14	0.433	4, 444	3.11	0.015
Iridescence	3, 4	7.31	0.042	4, 444	0.49	0.746
Yellow	3, 4	4.52	0.090	4, 444	0.76	0.553
Tail colour	3, 4	0.23	0.874	4, 444	11.29	0.000
Spot number	3, 4	11.22	0.020	4, 444	4.70	0.001

preferences, despite the widespread expectation that such a response should occur.

Lack of response in female preference

Statistical power

As the true level of replication in this kind of artificial selection experiment is the line, even a very large, long-term study such as ours may have low statistical power. This is even more the case when the measures involved are subject to large environmental variances via measurement error as is likely to be the case with our behavioural estimates of female preferences and attractiveness. Such environmental variance would result in low measured heritability of female preferences and male attractiveness and modest response to selection.

Low heritability

An obvious possible explanation for the lack of a significant selection response is that the heritabilities of male attractiveness and female mate choice may be low. Low heritabilities can be attributed either to a lack of additive genetic variation or to high levels of environmental variation [27]. If heritabilities are low, then the response to selection may be smaller than the minimal detectable difference for this experiment. Our finding that female preference for attractive males did not respond significantly to direct selection is consistent with estimates of zero heritability of this trait in this population under similar lab conditions [23]. In the study by Brooks and Endler [23], the heritability estimates of all components of mate choice (including responsiveness, discrimination and preferences for attractive males and various univariate male traits) were low and, in all cases other than responsiveness, not significantly greater than zero.

Both our result and the heritability estimates of Brooks and Endler [23] suggest that there might be a lack of additive genetic variation and / or abundant environmental variation, including measurement error, in mate choice (but see[16,19]).

Lack of response in male attractiveness

Low heritability

Although the same caveats about statistical power apply to male attractiveness, the possibility that male attractiveness failed to respond to direct selection due to a lack of additive genetic variation is less likely. By combining the selection intensity that we imposed with previous (significant) estimates of the heritability of male attractiveness in this guppy population ($h^2_{\text{sire}} = 0.596$, $h^2_{\text{sire\&dam combined}} = 0.348$, [24]) in the breeders' equation [27], we expected a change of 0.575 – 0.336 standard deviations in the

direction of selection on attractiveness. The small responses that did occur were inconsistent in direction and more than an order of magnitude smaller than the predicted responses. The observed responses were also at least three standard errors different from the predicted responses, indicating that we have the power to conclude that the predicted changes did not occur. The imposed selection intensities are comparable to those of other selection experiments on guppies. In the study by Houde [16], for example, a selection intensity of 0.386 per generation resulted in significant changes in male colouration. The selection intensities we imposed (mean 0.33 per generation) are also above the mean values documented in the wild (0.16, [28,29]).

Univariate accounts of phenotypic evolution are often inadequate. The evolution of a trait is influenced not only by its heritability and the intensity of selection operating directly upon it, but also by indirect selection when natural selection operates on genetically correlated traits [30]. Two recent studies have documented traits that fail to respond to selection as predicted by the univariate breeders' equation [31,32], and suggested that multivariate understanding of genetic variation and the operation of selection is necessary. We will now evaluate three possible multivariate explanations for why male attractiveness failed to respond as predicted to selection: complexity in the relationship between male attractiveness and the ornaments that underpin it, genetic constraint due to the patterns of genetic covariation among traits, and the "environmental covariance hypothesis".

Complex fitness surface

Blows, Brooks and Kraft [33] showed that in this population of guppies, the multivariate linear and nonlinear sexual selection fitness surface has at least three local fitness peaks corresponding roughly with large areas of orange colouration, of iridescence, and large tails. Consequently, there appear to be at least three ways in which male guppies can maximise attractiveness. Thus sexual selection on attractiveness and the ornaments that underpin it may not necessarily proceed in one direction. This is consistent with our observation that changes in ornamentation among lines and among treatments in our experiment occurred in different multivariate directions. Selecting on attractiveness, therefore, may have resulted in complex and inconsistent changes in ornamentation if different lines evolved toward different local optima in the fitness surface.

Genetic variance and covariance

There are two similar processes that may constrain the evolution of female preferences and male attractiveness. First, the genetic variation in and covariation among male ornaments that determine attractiveness are likely to influence the direction and rate of evolutionary change in male attractiveness. Second, trade-offs between attractiveness and other fitness components, including sexually antagonistic effects of genes influencing attractiveness may prevent any net change by sexual selection.

By selecting on male attractiveness, we imitated the direction of sexual selection in this population. The small responses of ornamental traits to selection on male attractiveness suggest that we may have selected in a direction that contains little multivariate genetic variation. Although there is abundant genetic variation in most ornamental traits in this guppy population, Brooks and Endler [15] demonstrated that most of this variation is not in the direction of sexual selection. By combining the genetic variance-covariance matrix, **G**, with the vector of estimated selection gradients, β, in the multivariate breeders equation [30,34], they predicted that one generation of sexual selection would result in changes of only 1–6% of one trait standard deviation. Thus the components of selection operating directly on ornamental traits are largely opposed by indirect selection on correlated traits, and multivariate genetic constraint on male ornamentation is a plausible explanation for the lack of response in attractiveness itself.

Selection acting on a trait in members of one sex may be constrained if there is antagonistic pleiotropy between the trait and fitness components in the opposite sex [35]. This is now known as intragenomic sexual conflict [36,37]. The continued exaggeration of male attractiveness, for example, may have been constrained by some costs to the expression of the female preference for attractive males [38] or negative genetic covariance between male attractiveness and important offspring fitness components [24,39].

In our study, female fecundity was significantly different between the four selection treatments. The most fecund treatment was the one in which we selected the least attractive males (UN), while the least fecund treatment was the one in which we selected on female preference for attractive males (PR). Furthermore, the eight line means of fecundity were significantly negatively correlated with line mean attractiveness ($r = -0.69$, $P < 0.05$), and with iridescence ($r = -0.93$, $P < 0.005$) and orange colouration ($r = -0.68$, $P < 0.05$) which are both key components of male attractiveness [15,33]. These results indicate that there may be a fecundity cost associated with male attractiveness and female preference for attractive males. The lack of response to selection may, therefore, be due to the fecundity selection that occurred within each selection line if females within the tank contributed unequally to the next generation. For example, although we selected for increased attractiveness, the least attractive males among

those selected may have benefited by having more fecund daughters than the most attractive males. Such a phenomenon would reduce the effective intensity of selection on attractiveness, causing any differences between treatments to fall below that the power our analyses could detect. The fact that we were able to detect the fecundity differences, yet did not observe any response to the selection is, however, paradoxical. The results do suggest that antagonistic evolution may be responsible in part for the lack of evolutionary change in female mate choice.

Other, similar, forms of natural selection within each line may also have constrained the response of male attractiveness to selection. Male attractiveness is negatively genetically correlated with the survival of juveniles and adult males [24], although we found that adult survival did not differ significantly between the selection treatments.

Environmental covariance hypothesis

Traits that are heritable and environmentally, but not genetically, correlated with fitness should not respond to selection – even though phenotypic selection analyses might predict such a response [31,40-42]. This "environmental covariance hypothesis" has been invoked to explain why antler size in the red deer, *Cervus elaphus*, did not respond to measured directional selection despite being highly heritable [31]. In this example, nutrition had independent positive effects on both fitness and antler size. Thus although fitness and antler size were positively correlated, this correlation was environmental rather than genetic (as would have been expected if large antlers caused higher fitness). A lack of evolutionary response is therefore attributed to a misidentified target of selection.

Like antler size, male attractiveness in the guppy is highly reliant on dietary condition. Orange colouration, for example, is a major component of attractiveness and partly derived from carotenoids in the diet of the guppy. Studies have also shown that increasing the carotenoid content of food will result in increased attractiveness [43,44]. It is conceivable, under the environmental covariance hypothesis that the commonly-documented relationship between orange colouration and attractiveness is due to an environmental correlation, and that sexual selection might thus have no net effect on the area orange colouration. This is unlikely, however, for two reasons. First, in the specific case of orange colouration, manipulative studies have shown that the relationship between levels of orange and attractiveness is causal: females prefer to mate with males because they directly assess the amount of orange in their colour pattern [45,46]. Second, male attractiveness and several components of male colouration are genetically correlated with one another and with mating success [15,24]. It appears unlikely, therefore, that the environmental covariance hypothesis can explain the lack of response by male attractiveness to selection.

Implications for the evolution of female mate choice

Our findings that neither male attractiveness nor female preference for attractive males responded significantly to three generations of selection (direct or indirect) have important implications for the evolution of mate choice by indirect selection. A long standing topic of controversy (the lek paradox) is whether and how sufficient additive genetic variation in male fitness and attractive ornamentation can be maintained in the face of selection to provide an indirect fitness benefit to choosy females [2,3]. Recent work has demonstrated both that considerable genetic variation in display traits exists [14] and suggested several possible processes that might maintain it [47-50].

Female guppies are thought to benefit from mating with attractive males because attractiveness itself is highly heritable and thus choosy females' sons are more likely to be attractive than are the sons of indiscriminate females [24]. The fact that in our study there was no measurable increment in male attractiveness (or in any measure of female choice) when we selected directly on male attractiveness indicates that the "attractive sons" benefit to choosy females may be smaller than one might infer from the univariate heritability of male attractiveness. This may be due to the fact that male attractiveness appears to be negatively genetically correlated with other fitness components, including male survival [24] and female fecundity (this study). It may also be due to the fact that male attractiveness is influenced by a suite of male traits, and that there is little multivariate genetic variation in the direction of selection despite the presence of additive genetic variation in each one of the male traits.

The indirect (genetic) benefits of mate choice can only arise if preferred males have higher breeding values for total fitness than non-preferred males [7]. It becomes clear from the issues that we have raised in this discussion that demonstrating heritable variation in one or a handful of attractive display traits is not sufficient evidence to dismiss the lek paradox. There is only one demonstration in any species that female preference is genetically correlated with offspring fitness [39], and no compelling demonstration that substantial genetic variation in offspring total fitness is correlated with variation in a trait that females use to choose mates. Tradeoffs and sexual antagonism between fitness components, and the possibility that little multivariate genetic variation in suites of ornaments is in the direction of sexual selection both raise the possibility of a new multivariate form of the lek paradox.

Methods

We collected guppies as juveniles from Alligator Creek, 30 km southwest of Townsville, Queensland. The use of wild-caught individuals to begin the selection lines ensures that naturally occurring genetic (co)variation is present. We raised the fish for the first selected (parental) generation in 100 litre single sex stock tanks.

We imposed three generations of selection on three selection treatments by selecting (1) directly up and (2) down on male attractiveness and (3) up on female preference for attractive males. We also conducted an unselected control. We use the following two letter abbreviations for the four types of line in the remainder of the manuscript: AT – Up attractiveness; UN – Down attractiveness; PR – Up preference; CO – Control. There were two replicates of each of the four types of line, but due to logistic constraints on the number of fish that we could maintain and measure, the replicate lines were conducted in two blocks. Each block contained one line from each of the four types. We performed the same experimental measures on each block but staggered the blocks by two months.

The dates on which we performed selection in each generation, and the number of individuals that we measured and selected are given in Table 1. In each generation, selected males and females from a line were placed into a 300 litre tank together to mate and produce offspring. This allows for sexual and other forms of natural selection to operate within lines at this stage, and has both advantages and disadvantages. An advantage over designs in which males and females are randomly paired and mated is that if mate choice is possible, linkage disequilibrium between male attractiveness and female preferences [1,6] may be maintained [51,52]. This disequilibrium is a crucial element of the genetic architecture of choice and attractiveness, and should be carefully considered when designing selection experiments. The disadvantages of our approach lie in the interpretation of any response (direct or correlated). Gray and Cade [53] point out that within-line sexual selection may cause an overestimate of the genetic correlation between preference and trait. This is not a problem in our study as we found no evidence of direct or indirect responses in these traits (see results). However, any selection on survival or fecundity within lines may either amplify or attenuate any response to the selection imposed by the researcher, a possibility we address in the discussion.

Offspring were collected daily and reared at initial densities of ten fry per six-litre tank. At approximately 40 days old, the fry were sexed based on the presence of female egg spots, and separated into single sex tanks. Tanks were covered on three sides with brown paper, and contained both floating and sessile plastic plants to provide refuge from harassment and (in the case of fry) cannibalism. Water was aerated and filtered using air-driven filters under a layer of light brown gravel. The temperature was kept constant at 26°C. A mixture of fluorescent and daylight lighting was used to illuminate the tanks. Throughout the experiment, we fed the fish five times a week on one-day old brine shrimp and twice a week on commercial flake food for tropical fish.

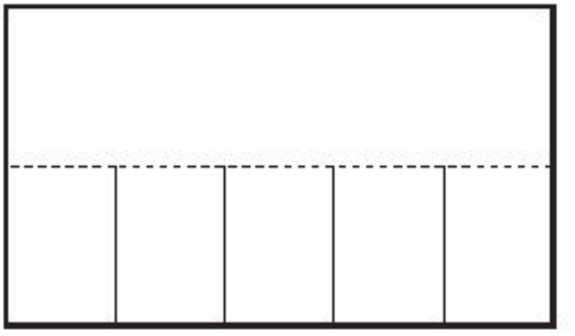

Figure 1
The choice tank used in measuring male attractiveness and female preference. Brown paper covered the side and back walls (bold line). Brown river sand covered the floor of tank. Scored glass separated (solid line) the five small compartments. Transparent glass (dotted line) separated the large and small compartments. Tank dimensions: 30 by 20 cm.

Selecting on attractiveness and preference

Our measures of male attractiveness and female preference were designed to capture these traits for the block as a whole so that preference-display runaways within selection lines do not obscure changes in the treatments relative to other lines within the block. Thus each male was seen by females from every treatment and likewise each female saw males from every treatment.

We measured male attractiveness and female mate choice in behavioural trials in partitioned-aquaria (Figure 1). We placed one male into each of the five small compartments, and a naive virgin focal female into the large compartment from where she could observe the five males. In the first generation of selection we randomly assigned one sixth of all males to the PR line, one sixth to the CO line, and one third to each of the AT and UN lines. Selection was applied (if at all) only to the individuals that had been assigned to the appropriate line. In the second and third generations, each choice tank contained one male from each line plus either an extra AT or UN male.

Up to twenty choice tanks were used per day during behaviour trials. Tanks were arranged over four rows, orientated towards an observer seated one meter away. During the behavioural trials, two daylight incandescent globes, placed behind the observer, provided lighting. All tanks experienced similar lighting intensities at the water surface (range 1.0–1.9 $\mu mol.m^{-2}.sec^{-1}$).

On the evening before a trial, we placed the five males and one female into each choice tank. Observations commenced the following morning between 0700 and 0800 hours and involved scanning all tanks consecutively fifty times. If a female was within one body length of and directly facing the compartment containing a male, we scored his compartment number. A male's attractiveness to a given female was the total number of such "visits" she paid him (maximum possible = 50). Similar partitioned-aquarium measures have been used extensively in studies of guppy mate choice, and attractiveness scores have been shown to significantly predict mating success [15,20]. We repeated the behavioural trial over five consecutive days, using a new focal female each day. On two of the five days the female was from the PR line, and the female was from each of the other three lines on one day each.

A male's attractiveness may be influenced by three factors: his actual attractiveness to the females that saw him, the choice tank he was in, and his location within the tank. It is important to control for the latter two factors. Typically, with the choice tank used in this experiment the outer two positions have elevated scores, followed by the next two positions (middle positions) and finally the centre position. To correct for the effect of position, we multiplied the scores of each male by a correction factor. We calculated the correction factor for each week of observations based on the average score recorded at each position in all tanks on all five days in that week.

A preference function is how a female ranks prospective mates based on a specific male trait [54]. A female's "preference for attractive males" indicates the extent to which a female's choices are consistent with those of her peers [23]. We estimated a female's preference for attractive males as the slope coefficient of the least-squares regression of how she rated the five males on the mean scores that those males received from the other four females who saw them. A positive slope indicates that a female rated the males in the same way as the other four females, and thus presumably the majority of the population. Furthermore, the larger the positive slope the more strongly the female of interest responded to attractive males. The use of linear regression gradients as estimates of the strengths of selection is valid irrespective of whether the assumptions of linear regression significance tests (e.g. normality) are met [30]. This method is, however, prone to error because slopes were estimated from only five data points. Furthermore, although there is no autocorrelation in the estimated slopes, the use of the mean of four females' scores as the independent variable (attractiveness) means that there is some nonindependence to the preference estimates within a trial. This nonindependence did not, however, result in significant resemblance between the preference measures taken within a tank in a given week (ANOVA $F_{36,395} = 1.184$, $P = 0.221$).

Terminal measures

In the F3 generation, we measured a suite of traits to estimate the direct response of each selected trait and any correlated responses in other potentially correlated traits. We measured male attractiveness, female mate choice, male ornamentation, survival and fecundity. A total of 57 virgin males and 107 virgin females from each selection line per block were used for these terminal measures.

We used two different types of behavioural trials to measure female mate choice and male attractiveness. First, we conducted partitioned-aquaria behavioural trials (as in the selection process) which allow for individual identification of each focal female without direct interactions between males and females. We then used open-aquarium behavioural trials, in which males and females can interact freely and the full range of male courtship and female response behaviours can occur [20]. In the partitioned-aquaria trials, male attractiveness and female preference for attractive males were measured as described above for selection. We estimated a female's responsiveness as the number of times she was seen with any of the males and discrimination as the coefficient of variation in her number of visits to the five males in the tank.

The open-aquarium behavioural trials were conducted in 100 L aquaria under the same lighting conditions as in the partitioned-aquaria trials. On the night before observations, we placed eight males and eight females into the behavioural tank. Each set of eight males contained two males from each selection treatment. The females on any given day were all from the same selection treatment. We used eight new males and eight new females on each day. Males were individually identified by the observer from their unique colour patterns.

Observations began between 0700 and 0800 hours. We watched each male for a five-minute period in random order, and then for a second five-minute period each in a different random order. Finally, we spent ten minutes scanning the tank, shifting from one male to the other approximately every 30 seconds, to ensure that we observed a total of at least five displays per male. We followed the standard procedures of Houde [20,21] in scoring a male's attractiveness as the proportion of his

sigmoid displays that elicited at least a "glide" response from a female. We measured female responsiveness as the mean response of females to all males in the trial. Only a single measure of responsiveness could be obtained for each trial, as females cannot be individually distinguished.

We photographed the right side of each male against a white background with a Nikon Coolpix 990 digital camera, including a ruler with millimetre graduations in the picture for calibration. Each male was anaesthetised beforehand with iced water and illuminated dorsally and anteriorly (30° angle of incidence) with low intensity halogen light (Fostec ACE 150 watt light source). We then traced the area of the body, the tail and each colour spot using Measure Master (Version 3.4) digital imaging analysis software. From the tracings, we calculated body area and tail area, and the proportion of the body covered by black, fuzzy black, orange, yellow and iridescent spots. We also counted the of coloured spots on his body.

We conducted short-term adult survival and fecundity trials by placing 50 males from one line (used in the previous attractiveness measures) and 50 naive virgin females from the same line into a 250 litre mating tank. Each day for the next 60 days we collected and counted the number of offspring produced. At the end of the 60-day trial we recorded the number of adults of each sex remaining.

Statistical analysis

We standardised the measures of traits by block means and standard deviations in order to control for environmental variation among blocks. We then assessed differences between the treatments using nested analysis of variance and one-way analysis of variance.

We estimated realized heritabilities of directly selected traits by applying the standardized form of the breeder's equation (Equation 11.3, ref [27]). We estimated the response to selection as the difference between the line means for each trait of interest in the **F3** generation and the corresponding block's control line means. We then standardised the response to be in units of the control line phenotypic standard deviation for the trait of interest. By standardising the response, we are able to use the intensity of selection, *i* (Equation 11.5, ref [27]), instead of the selection differential, *s*, which was considerably more difficult to calculate in this experiment. We calculated *i* based on the proportion selected and properties of normal distributions (Appendix A, ref [27]) and modified our estimate based on the ratio of the selected sex to the other sex (Equation 11.6b, [27]). We then calculated realized heritabilities from the standardized breeders' equation. Each replicate selection line provides one estimate of the realized heritability, allowing a mean and standard error to be directly estimated for each selection treatment.

Author's contributions

RB conceived and initiated the study. All authors measured behaviour, conducted selection and contributed to the design of the terminal measures. MH undertook the terminal measures, analysed the data and wrote a first draft of the manuscript. All authors contributed to the final analyses and writing of the manuscript.

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References

1. Fisher RA: *The Genetical Theory of Natural Selection. Oxford, Oxford University Press.*; 1930.
2. Kirkpatrick M, Ryan MJ: **The evolution of mating preferences and the paradox of the lek.** *Nature* 1991, **350:**33-38.
3. Andersson M: *Sexual Selection Princeton, N.J., Princeton University Press*; 1994.
4. Kokko H, Brooks R, Jennions MD, Morley J: **The evolution of mate choice and mating biases.** *Proc R Soc Lond B* 2003, **270:**653-664.
5. Pomiankowski A: **The costs of choice in sexual selection.** *Journal of Theoretical Biology* 1987, **128:**195-218.
6. Lande R: **Models of speciation by sexual selection on polygenic traits.** *Proc Nat Acad Sci USA* 1981, **78:**3721-3725.
7. Kokko H, Brooks R, McNamara JM, Houston AI: **The sexual selection continuum.** *Proc R Soc Lond B* 2002, **269:**1331-1340.
8. Eshel I, Volovik I, Sansone E: **On Fisher-Zahavi's handicapped sexy son.** *Evolutionary Ecology Research* 2000, **2:**509-523.
9. Kirkpatrick M: **Good genes and direct selection in the evolution of mating preferences.** *Evolution* 1996, **50:**2125-2140.
10. Kirkpatrick M, Barton NH: **The strength of indirect selection on female mating preferences.** *Proc Nat Acad Sci USA* 1997, **94:**1282-1286.
11. Houle D, Kondrashov AS: **Coevolution of costly mate choice and condition-dependent display of good genes.** *Proc R Soc Lond B* 2002, **269:**97-104.
12. Darwin C: **Heritabilities and paradigm shifts.** *The Descent of Man and Selection in Relation to Sex London: Murray*; 1871.
13. Alatalo RV, Mappes J, Elgar MA: **Heritabilities and paradigm shifts.** *Nature* 1997, **385:**402-403.
14. Bakker TCM: **The study of intersexual selection using quantitative genetics.** *Behaviour* 1999, **136:**1237-1265.
15. Brooks R, Endler JA: **Direct and indirect sexual selection and quantitative genetics of male traits in guppies (Poecilia reticulata).** *Evolution* 2001, **55:**1002-1015.
16. Houde AE: **Effect of artificial selection on male colour patterns on mating preference of female guppies.** *Proc R Soc Lond B* 1994, **256:**125-130.
17. Wilkinson GS, Reillo PR: **Female choice response to artificial selection on an exaggerated male trait in a stalk-eyed fly.** *Proc R Soc Lond B* 1994, **255:**1-6.
18. von Schantz T, Tufvesson M, Göransson G, Grahn M, Wilhelmson M, Wittzell H: **Artificial selection for increased comb size and its effects on other sexual characters and viability in Gallus domesticus (the domestic chicken).** *Heredity* 1995, **75:**518-529.
19. Brooks R, Couldridge V: **Multiple Sexual Ornaments Coevolve with Multiple Mating Preferences.** *Am Nat* 1999, **154:**37-45.
20. Houde AE: *Sex, Color and Mate Choice in Guppies Princeton, N.J., Princeton University Press*; 1997.
21. Houde AE: **Mate choice based upon naturally occurring colour-pattern variation in a guppy population.** *Evolution* 1987, **41:**1-10.
22. Breden F, Hornaday K: **Test of indirect models of selection in the Trinidad guppy.** *Heredity* 1994, **73:**291-297.
23. Brooks R, Endler JA: **Female guppies agree to differ: phenotypic and genetic variation in mate-choice behaviour and the**

consequences for sexual selection. *Evolution* 2001, **55:**1644-1655.
24. Brooks R: **Negative genetic correlation between male sexual attractiveness and survival.** *Nature* 2000, **406:**67-70.
25. Houde AE: **Sex-linked heritability of a sexually selected character in a natural population of Poecilia reticulata (Pisces: Poeciliidae) (guppies).** *Heredity* 1992, **69:**229-235.
26. Hall DW, Kirkpatrick M, West B: **Runaway sexual selection when female preferences are directly selected.** *Evolution* 2000, **54:**1862-1869.
27. Falconer DS, Mackay TFC: *Introduction to Quantitative Genetics, 4th edn. New York, Longman*; 1996.
28. Hoekstra HE, Hoekstra JM, Berrigan D, Vignieri SN, Hoang A, Hill CE, Beerli P, Kingsolver JG: **Strength and tempo of directional selection in the wild.** *Proc Nat Acad Sci USA* 2002, **98:**9157-9160.
29. Kingsolver JG, Hoekstra HE, Hoekstra JM, Berrigan D, Vignieri SN, Hill CE, Hoang A, Gibert P, Beerli P: **The strength of phenotypic selection in natural populations.** *Am Nat* 2001, **157:**245-261.
30. Lande R, Arnold SJ: **The measurement of selection on correlated characters.** *Evolution* 1983, **37:**1210-1226.
31. Kruuk LEB, Slate J, Pemberton JM, Brotherstone S, Guinness FE, Clutton-Brock TH: **Antler size in red deer: heritability and selection but no evolution.** *Evolution* 2002, **56:**1683-1695.
32. Merilä J, Kruuk LEB, Sheldon BC: **Cryptic Evolution in a wild bird population.** *Nature* 2001, **412:**76-79.
33. Blows MW, Brooks R, Kraft PG: **Exploring complex fitness surfaces: multiple ornamentation and polymorphism in male guppies.** *Evolution* 2003, **57:**1622-1630.
34. Lande R: **Quantitative genetical analysis of multivariate evolution, applied to brain:body size allometry.** *Evolution* 1979, **33:**402-416.
35. Lande R: **Sexual dimorphism, sexual selection, and adaptation in polygenic characters.** *Evolution* 1980, **34:**292-305.
36. Chippendale AK, Gibson JR, Rice WR: **Negative genetic correlation for adult fitness between sexes reveals ontogenetic conflict in Drosophila.** *Proc Nat Acad Sci USA* 2001, **98:**1671-1675.
37. Rice WR, Chippendale AK: **Intersexual ontogenetic conflict.** *J evol Biol* 2001, **14:**685-693.
38. Price DK, Burley NT: **Constraints on the evolution of attractive traits: selection in male and female zebra finches.** *Am Nat* 1994, **144:**908-934.
39. Hine E, Lachish S, Higgie M, Blows MW: **Positive genetic correlation between female preference and offspring fitness.** *Proc R Soc Lond B* 2002, **692:**2215-2219.
40. Rausher MD: **The measurement of selection on quantitative traits: biases due to environmental covariances between traits and fitness.** *Evolution* 1992, **46:**616-626.
41. Stinchcombe JR, Rutter MT, Burdick DS, Tiffin P, Rausher MD, Mauricio R: **Testing for environmentally induced bias in phenotypic estimates of natural selection.** *Am Nat* 2002, **160:**511-523.
42. Kruuk LEB, Merilä J, Sheldon BC: **When environmental variation short-circuits natural selection.** *Trends in Ecology and Evolution* 2003, **18:**207-209.
43. Grether GF: **Carotenoid limitation and mate preference evolution: a test of the indicator hypothesis in guppies.** *Evolution* 2000, **54:**1712-1724.
44. Grether GF, Hudon J, Endler JA: **Carotenoid scarcity, synthetic pteridine pigments and the evolution of sexual colouration in guppies (Poecilia reticulata).** *Proc R Soc Lond B* 2001, **268:**1245-1253.
45. Long KD, Houde AE: **Orange spots as a visual cue for female mate choice in the guppy (Poecilia reticulata).** *Ethology* 1989, **82:**316-324.
46. Brooks R, Caithness N: **Female guppies use orange as a mate choice cue: a manipulative test.** *South African Journal of Zoology* 1995, **30:**200-201.
47. Rowe L, Houle D: **The lek paradox and the capture of genetic variance by condition dependent traits.** *Proc R Soc Lond B* 1996, **263:**1415-1421.
48. Kotiaho JS, Simmons LW, Tomkins JL: **Towards a resolution of the lek paradox.** *Nature* 2001, **410:**684-686.
49. Pomiankowski A, Iwasa Y, Nee S: **the evolution of costly mate preferences I. Fisher and biased mutation.** *Evolution* 1991, **45:**1422-1430.
50. Hamilton WD, Zuk M: **Heritable true fitness and bright birds: A role for parasites?** *Science* 1982, **218:**384-387.
51. Pomiankowski A, Sheridan L: **Linked sexiness and choosiness.** *Trends in Ecology and Evolution* 1994, **9:**242-244.
52. Bakker TCM, Pomiankowski A: **The genetic basis of female mate preferences.** *J evol Biol* 1995, **8:**129-171.
53. Gray DA, Cade WH: **Correlated-response-to-selection experiments designed to test for a genetic correlation between female preferences and male traits yeild biased results.** *Anim Behav* 1999, **58:**1325-1327.
54. Jennions MD, Petrie M: **Variation in mate choice and mating preferences: a review of causes and consequences.** *Biol Rev* 1997, **72:**283-327.